From protein Scissors to Enzyme-Inorganic Materials: Biomolecular Recognition

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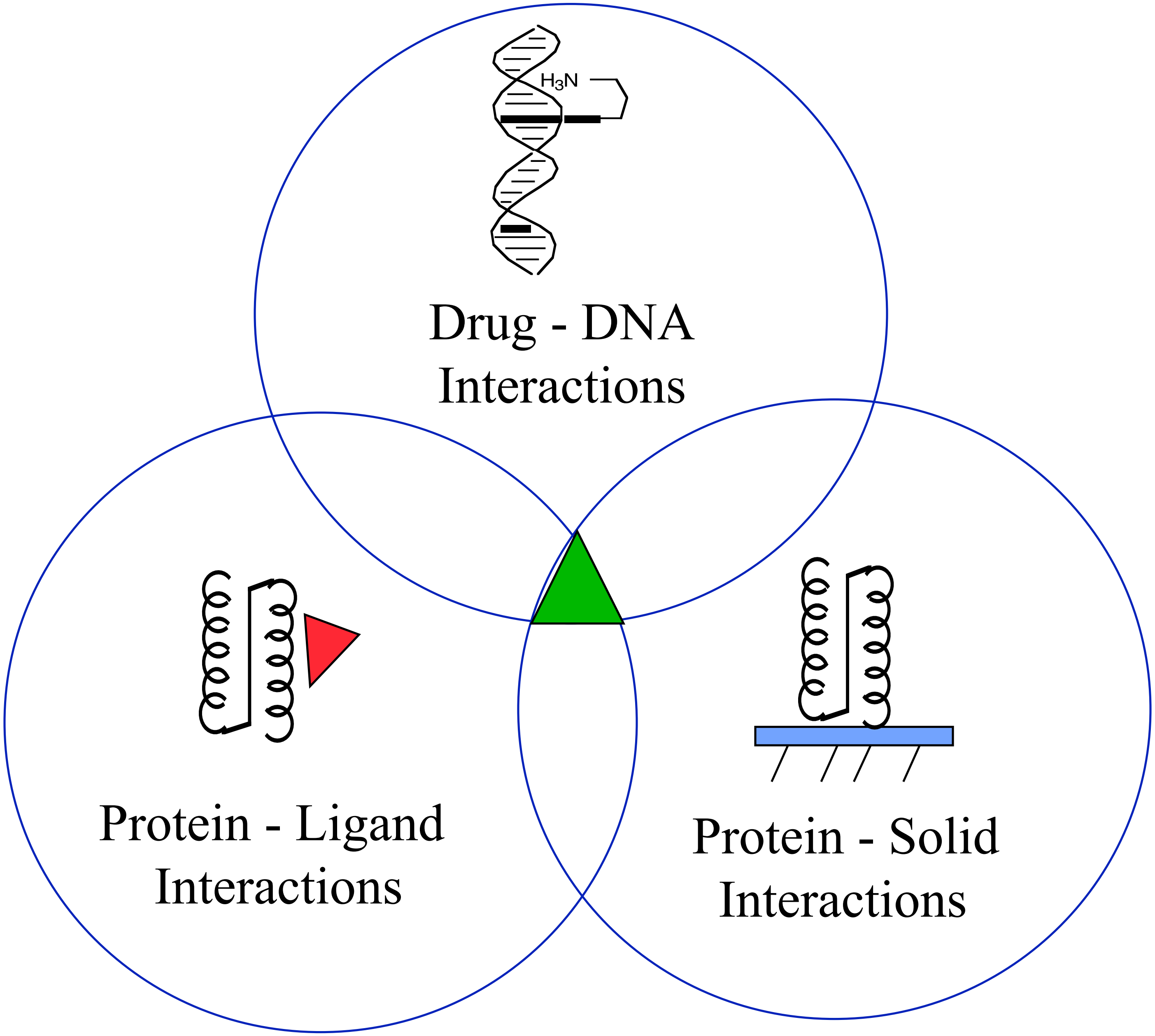
Structure Recognition: Non-covalent Interactions

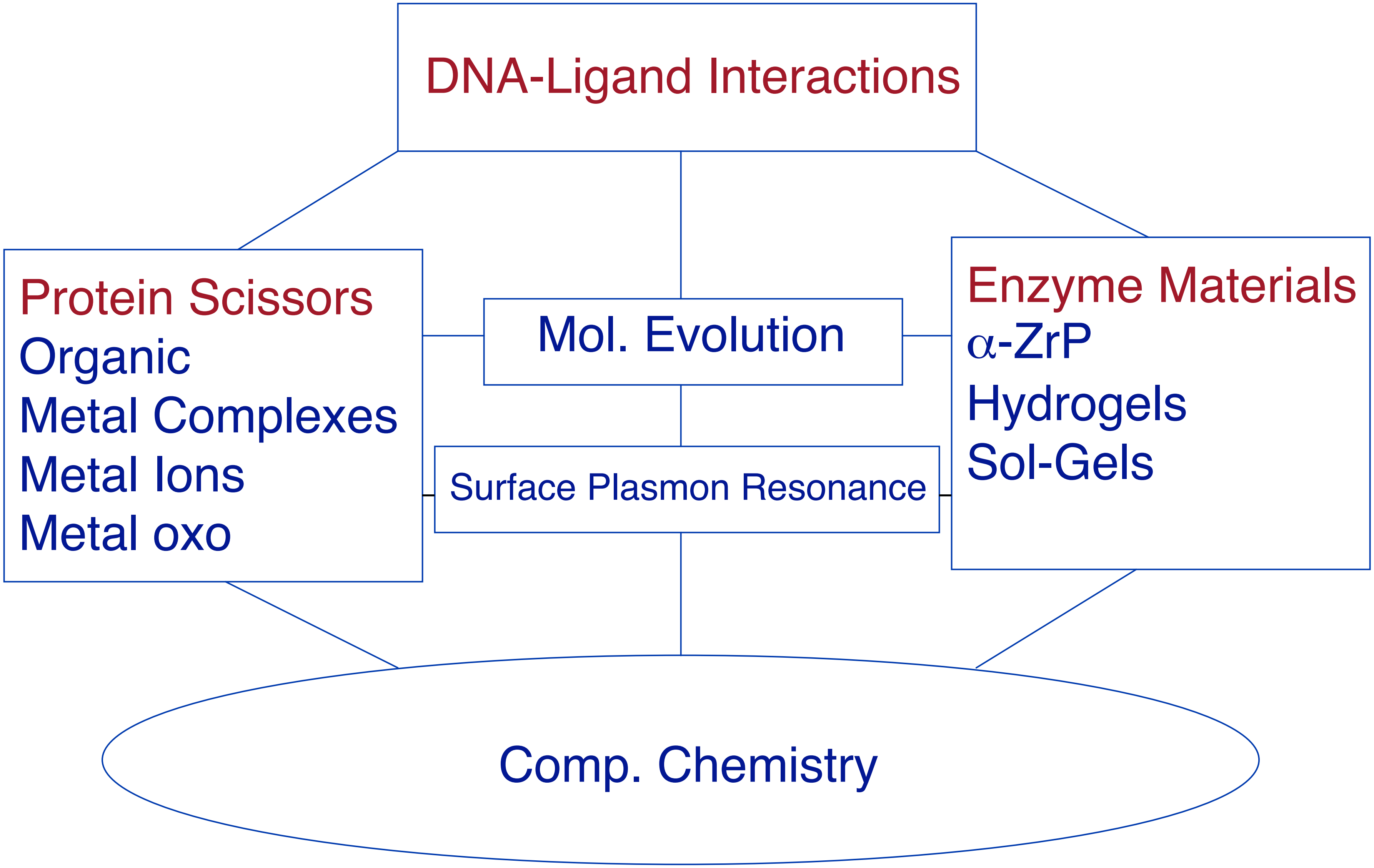


Drug - DNA
Interactions

Protein - Ligand
Interactions

Protein - Solid
Interactions

Biomolecular Structure Recognition



DNA-Ligand Interactions

Protein Scissors
Organic
Metal Complexes
Metal Ions
Metal oxo

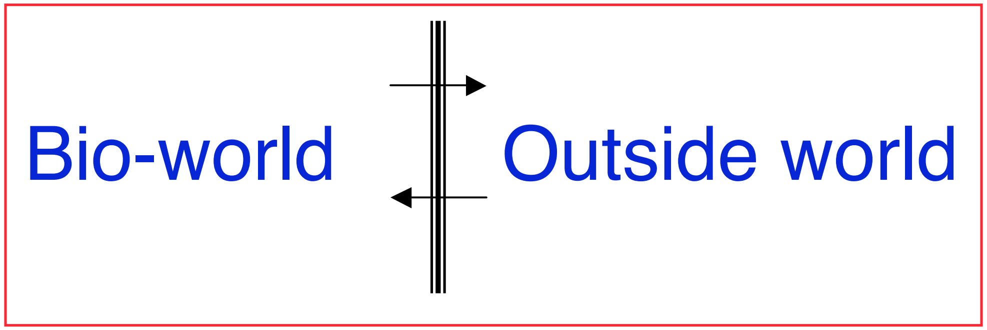
Mol. Evolution

Surface Plasmon Resonance

Enzyme Materials
α-ZrP
Hydrogels
Sol-Gels

Comp. Chemistry

Motivation


Bio-world

Outside world

Structure Recognition is important in:

Therapy: Targeting specific biomolecules.. ..

Diagnostics: Biomolecular detection….

1. Biosensors; Implants (pace-makers, insulin pumps…)
2. Prosthetics (smart limbs)
3. Artificial vision
4. Induced limb movement (paralysis victims)
5. Biochips for disease identification
6. Biocatalysis with enzymes/organelles/cells

Protein Scissors

Reagents that can cleave proteins at specific sites

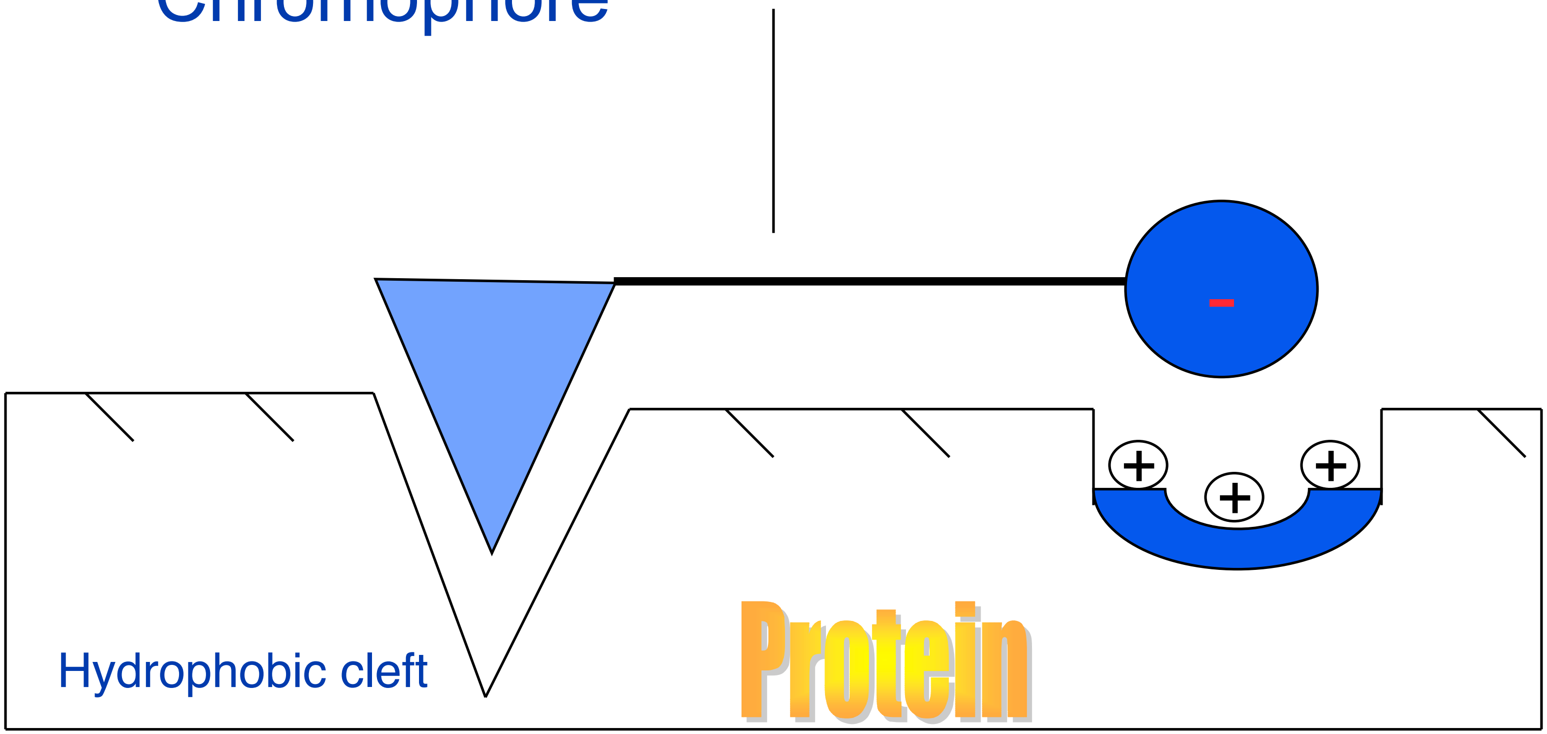
Photochemical (organic/metal complexes/Metal Oxo)

Oxidative (Metal ions)

Hydrolytic (Metal complexes)

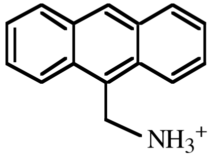
De Novo Design: Modular Approach

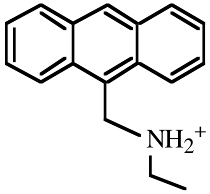
Photoactive ----Linker---- Ionic group
Chromophore

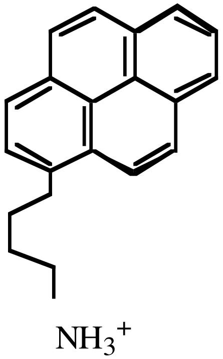


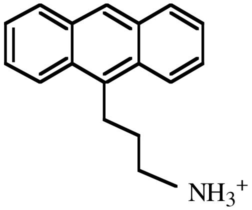
Multiple, Dissimilar Interactions: Key for Recognition

First-Generation Scissors









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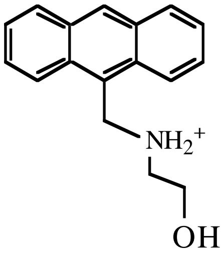
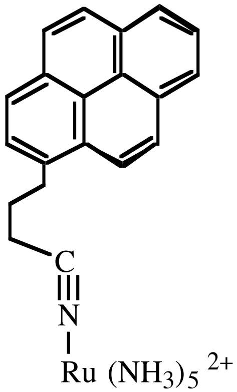
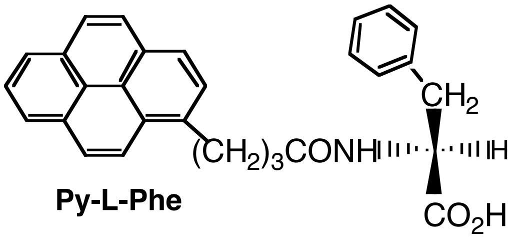




Longer linkers resulted in better binding constants

J. Phys. Chem., 1993, *87*, 13914

2nd Generation Scissors



Py-L-Phe

Py-Phe

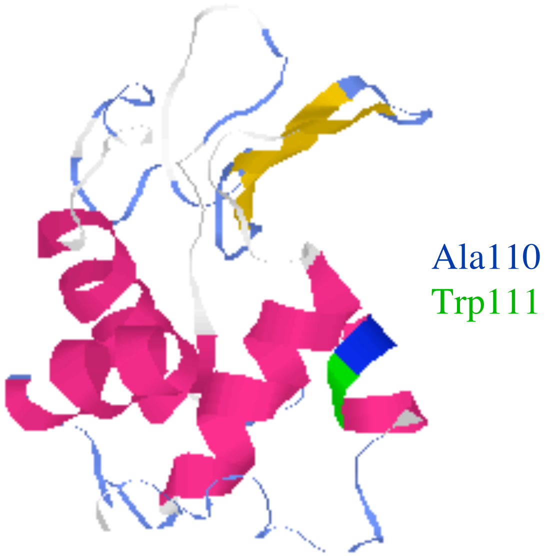
Py-Gly

Py-Phe-Gly

Py-Gly-Phe

1. Solid phase synthesis is rapid.
2. Large quantities are made easily.
3. Multiple chiral centers are readily introduced.
4. Modules can be varied systematically.

Binding Studies

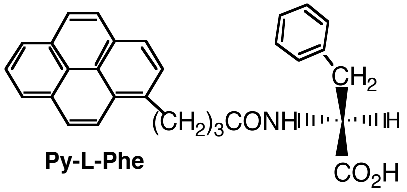


Model Proteins:

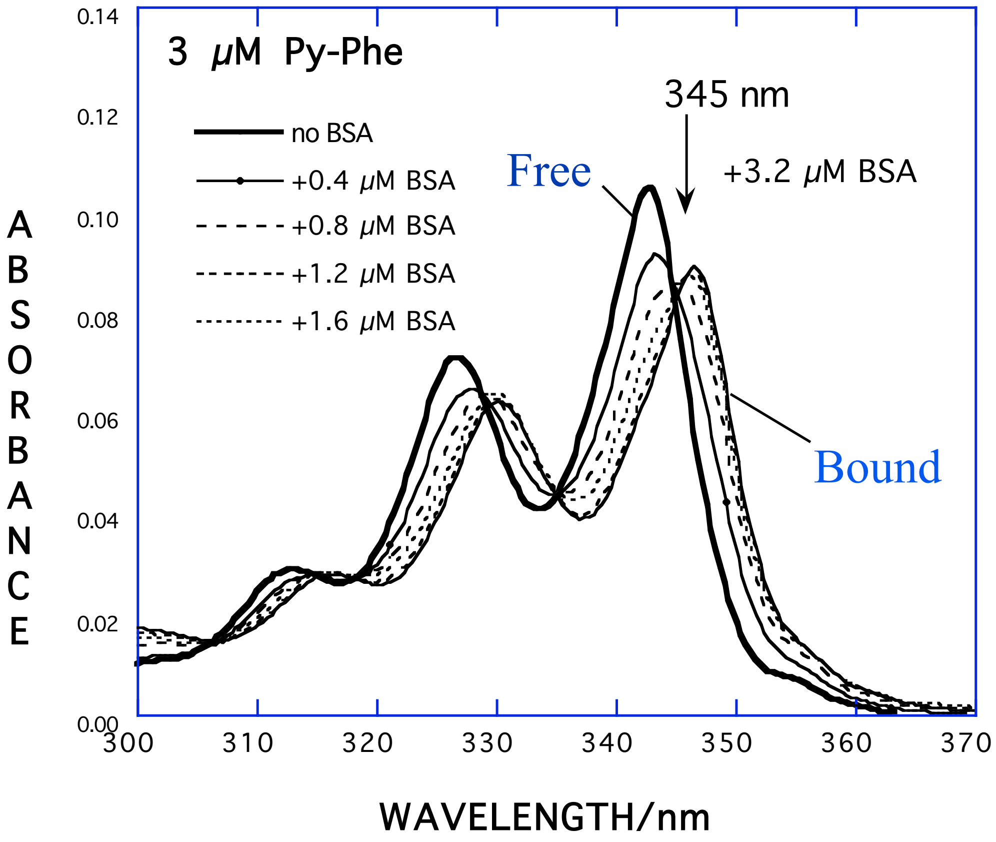
Bovine Serum Albumin (BSA, 584 residues, major plasma protein)

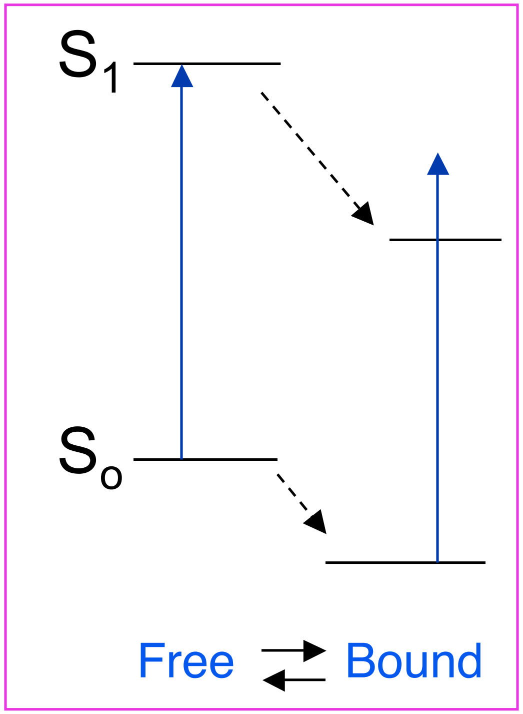
Lysozyme (129 residues, hydrolytic enzyme, pI=11)

Absorption Titrations (Py-Phe/BSA)

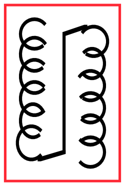


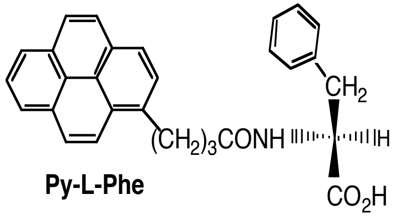
Py-L-Phe

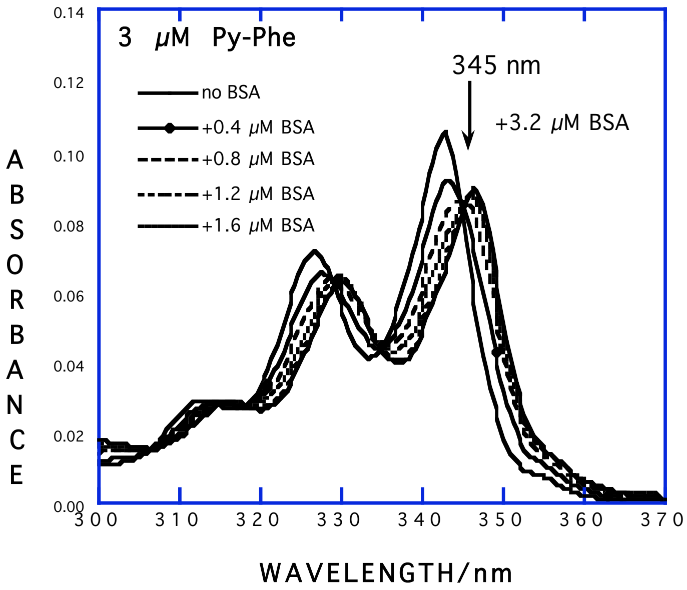




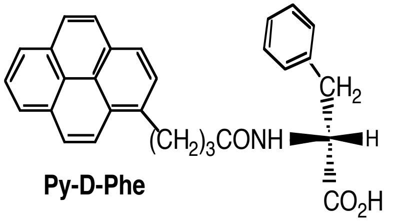
Chiral Recognition with BSA

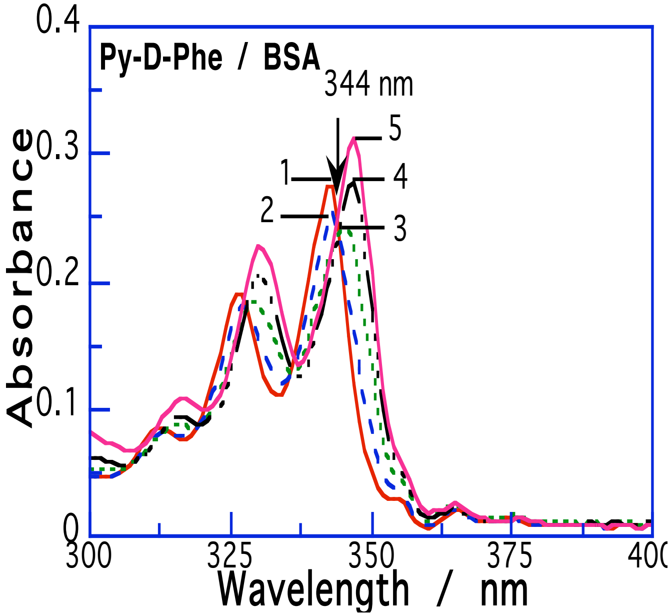



Py-L-Phe



$K_b = 6.5x10^7/M$


Py-D-Phe



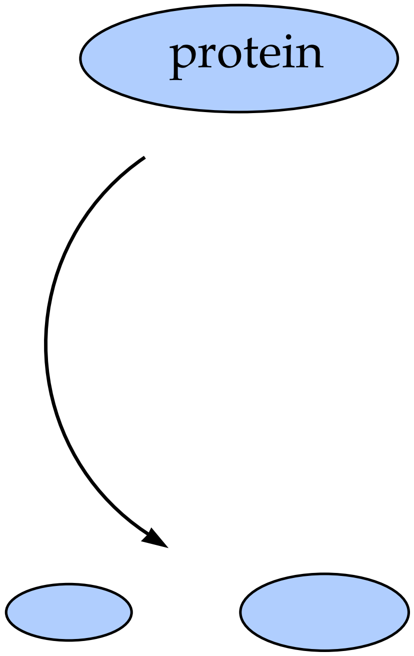
$K_b = 2.2x10^5/M$

Chem. Comm., 2001, 297

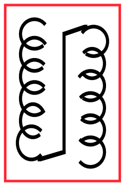
Protein Photocleavage

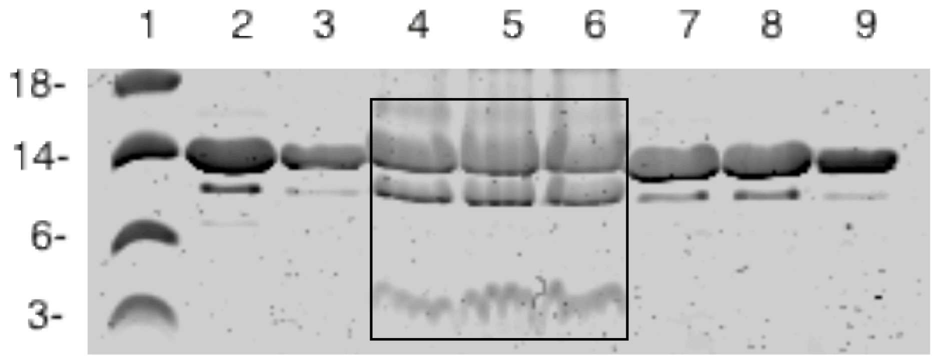
Electron Acceptor

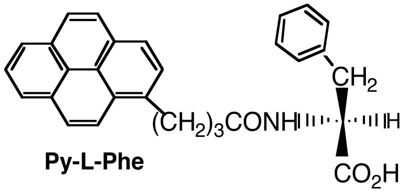
$$\text{Py-Phe} \xrightarrow{h\nu} {}^{1}(\text{Py-Phe})^{*} \xrightarrow[\text{- CoHA}^{-}\bullet]{\text{CoHA}} \text{Py-Phe}^{+}\bullet$$



Photocleavage of Lysozyme (15 μM) Py-Phe (15 μM)

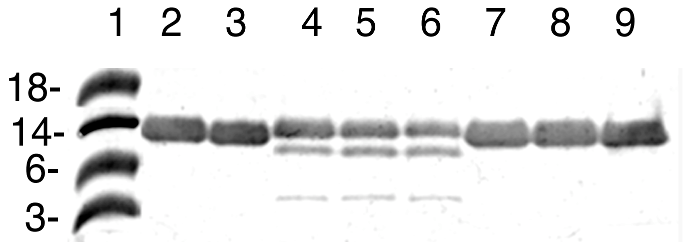






Py-L-Phe

1. Mol. wt. markers (kD)
2. Lysozyme
3. Lysozyme + Py-Phe + 1 mM CoHA, No Light
4. Lysozyme + Py-Phe + 1 mM CoHA, Light, 5 min.
5. Lysozyme + Py-Phe + 1 mM CoHA, Light, 10 min.
6. Lysozyme + Py-Phe + 1 mM CoHA, Light, 20 min.
7. Lysozyme + Light, 20 min.
8. Lysozyme + Py-Phe, Light, 20 min.
9. Lysozyme + CoHA, Light, 20 min.

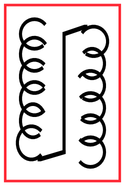


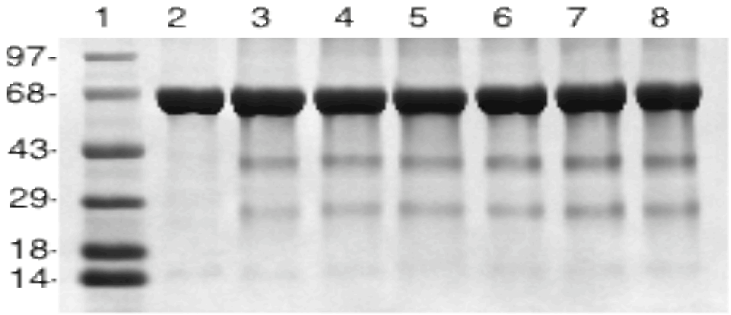
First example of photocleavage of proteins by an organic molecule!

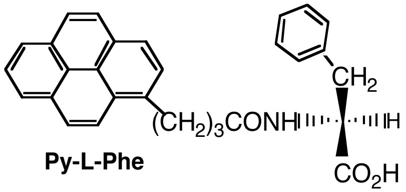
Chem. & Engg. News 1998

Photocleavage of Bovine Serum Albumin

BSA (15 μM), Py-Phe (15 μM)



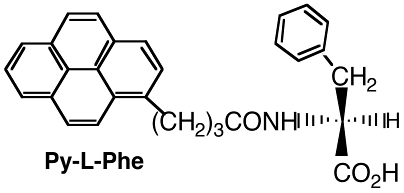




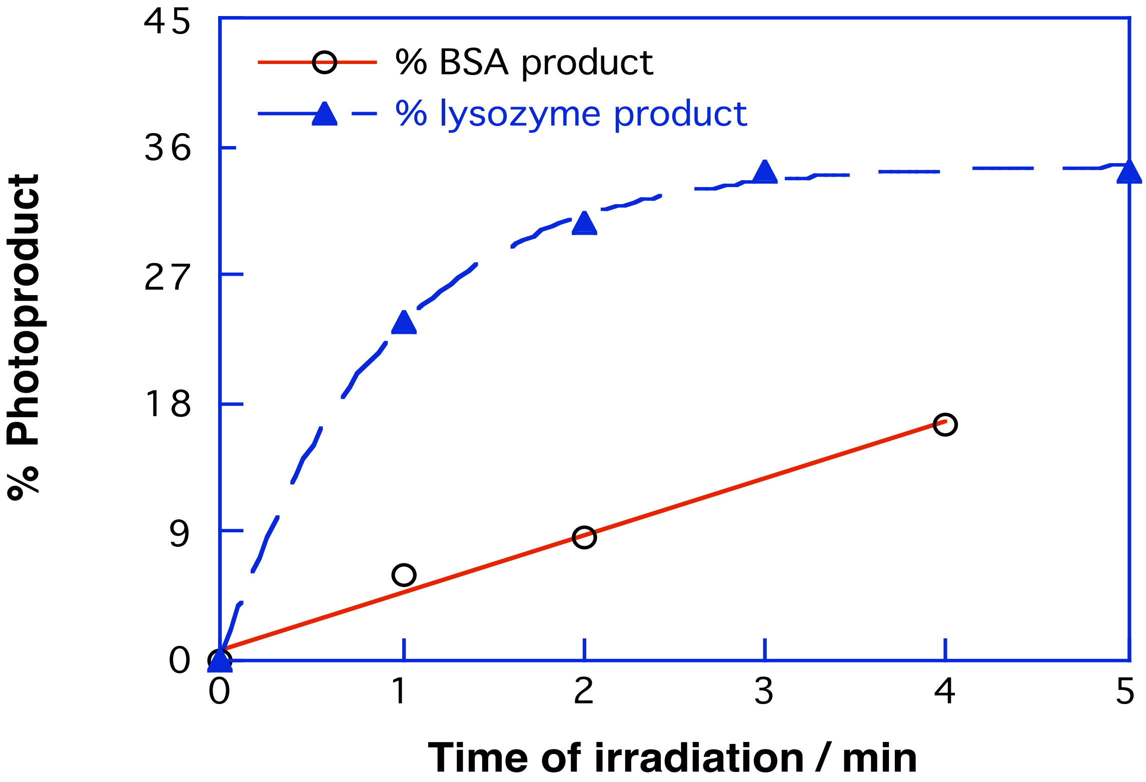
1. M. Wt. Standard (kDa)
2. BSA
3. BSA + Py-Phe + CoPA, 20 min
4. BSA + Py-Phe + CoPA, 40 min
5. BSA + Py-Phe + CoPA, 60 min
6. BSA + Py-Phe + CoHA, 20 min
7. BSA + Py-Phe + CoHA, 40 min
8. BSA + Py-Phe + CoHA, 60 min

Angew. Chem., 1997, *36*, 205

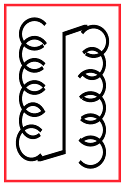
Photocleavage Progress with Time

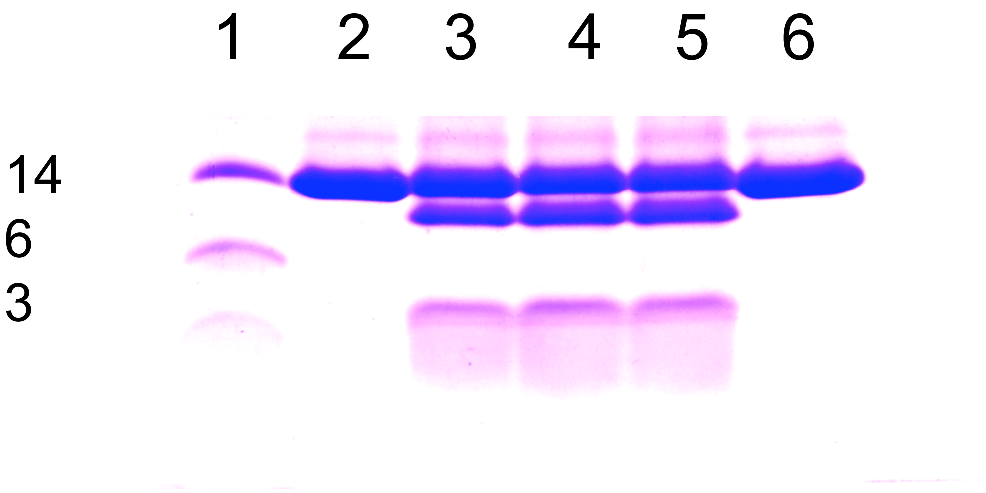


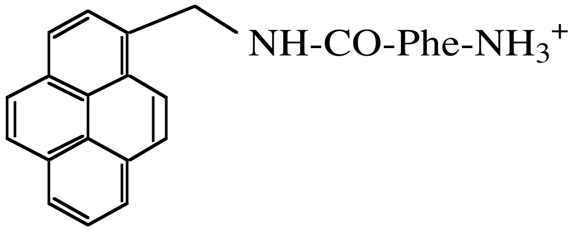
Py-L-Phe



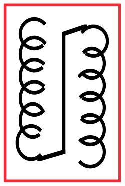
Photocleavage of lysozyme by PMA-Phe







1: M.W. markers (kD)
2: 15 µM lysozyme + 15 µM PMA-Phe + 1 mM CoHA , Dark control
3: 15 µM lysozyme + 15 µM PMA-Phe + 1 mM CoHA , Light 10 min (54%)
4: 15 µM lysozyme + 15 µM PMA-Phe + 1 mM CoHA, Light 20 min (56%)
5: 15 µM lysozyme + 15 µM PMA-Phe + 1 mM CoHA, Light 30 min (57%)
6: 15 µM lysozyme + 15 µM PMA-Phe, Light 30 min
Irradiation at 342 nm



Lysozyme Cleavage Site (Py-Phe)

108 Residues, N-Terminal Pepetide

21 Residues C-Terminal Peptide

H_2N-LysValPhe.................AlaTrp - ValAlaTrpArg..................Leu-COOH

108 109

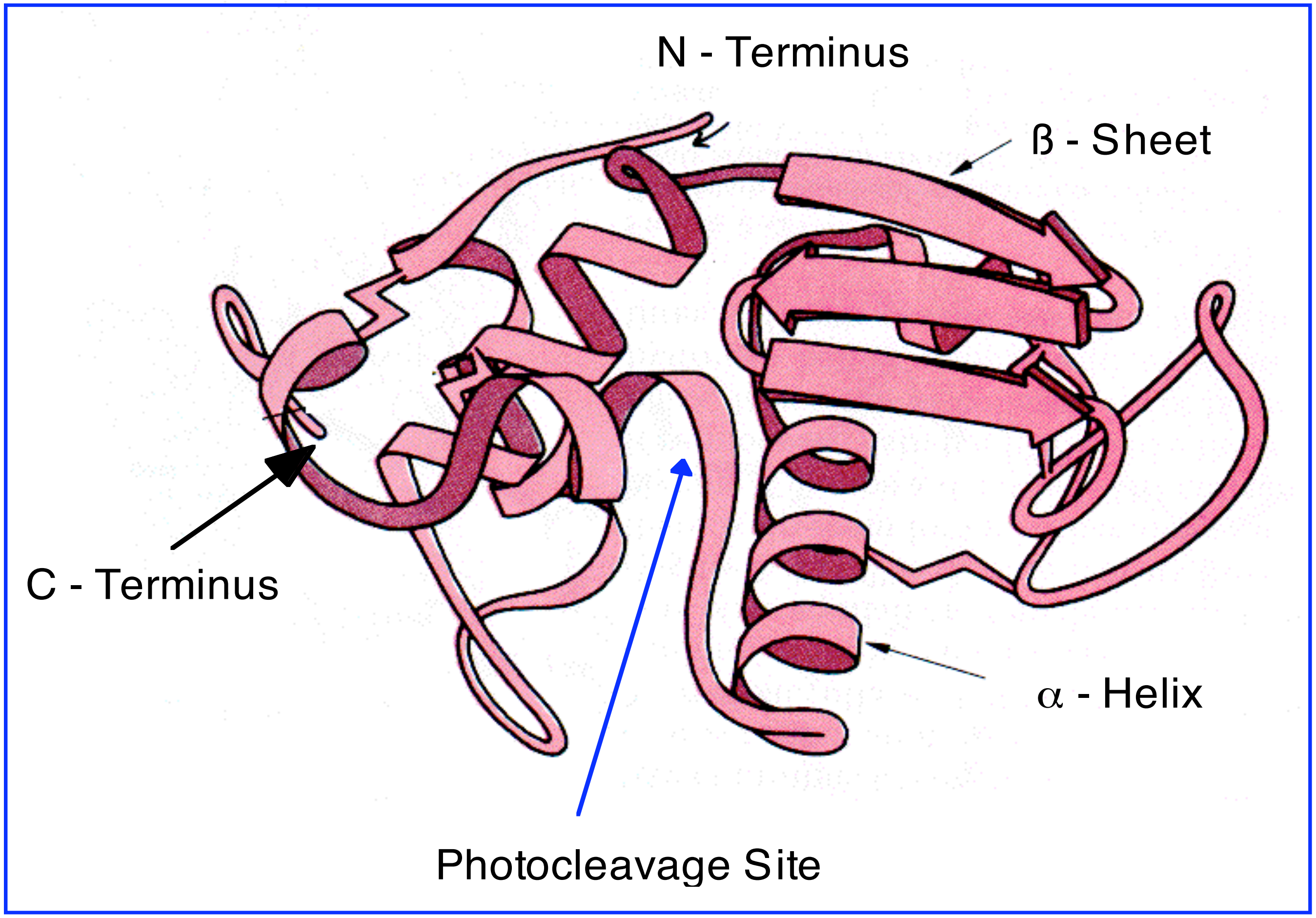
11460 Da

3057 Da

Cleaves at a single site out of 128 possibilities!

PNAS, 1998

Lysozyme Cleavage Site



Trp108-Val109

Bovine Serum Albumin Cleavage Site (Py-Phe)

346 Residues, N-terminal Peptide

238 Residues, C-Terminal Peptide

H_2N-AspThr...............ValLeuLeu - ArgLeuAla..................Ala-COOH

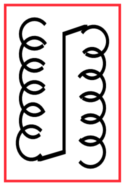
346 347

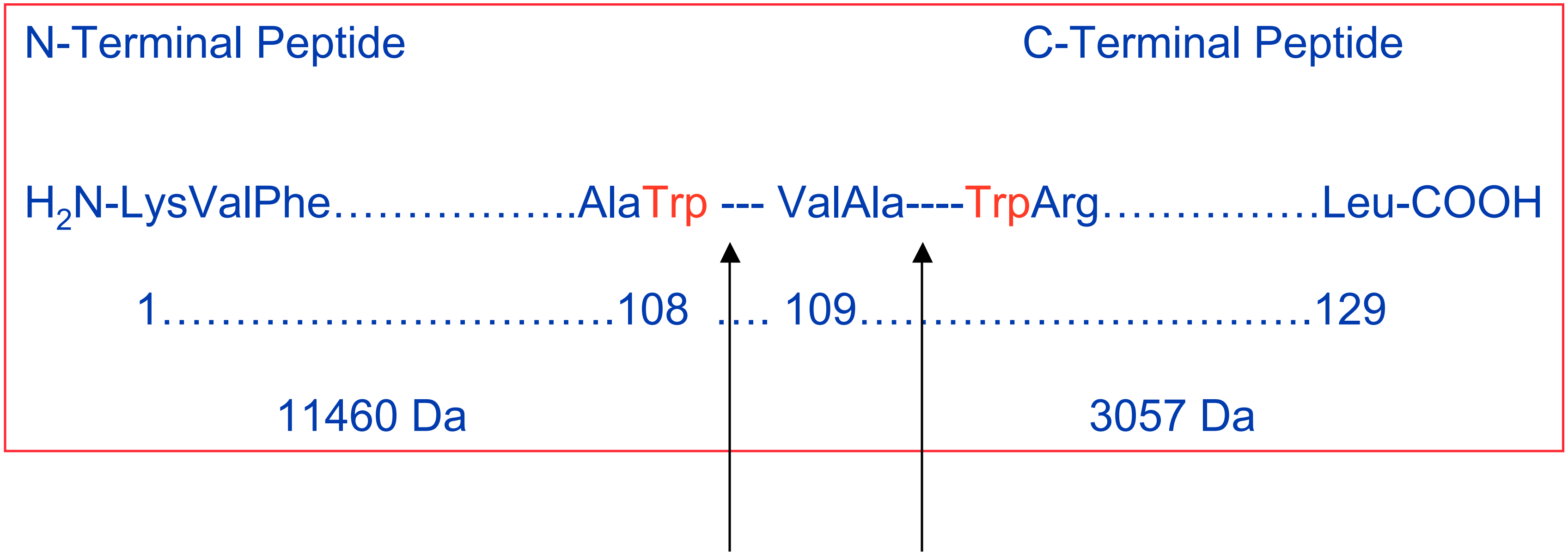
39930 Da

26860 Da

Hits a single site out of ~580 possibilities.

Lysozyme Cleavage Site (Py-Gly-Gly-Phe)





N-Terminal Peptide

C-Terminal Peptide

H_2N-LysValPhe……………..AlaTrp --- ValAla----TrpArg……………Leu-COOH

1……………………………108 …. 109…………………………….129

11460 Da

3057 Da

Py-Phe 1 : 0

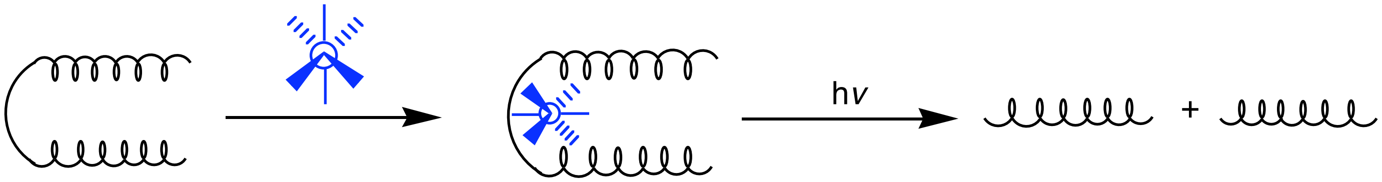
Py-Gly-Phe 6 : 1

Py-Gly-Gly-Phe 6 : 1

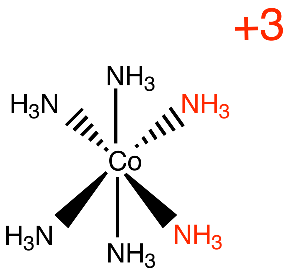
Py-Phe-Gly-Gly 4 : 1

JACS 2000

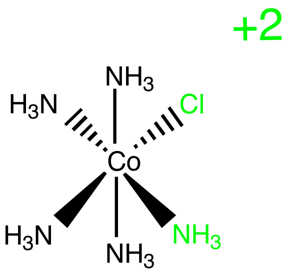
Photocleavage of Proteins by Co(III) Complexes



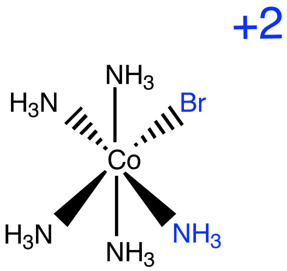
Strategy



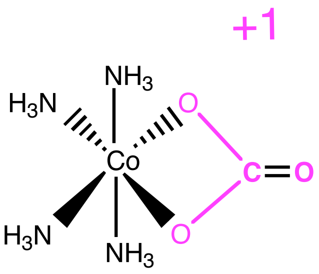
CoHA



CoPACl

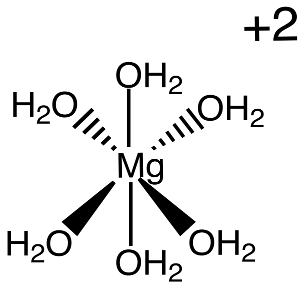


CoPABr



CoTA

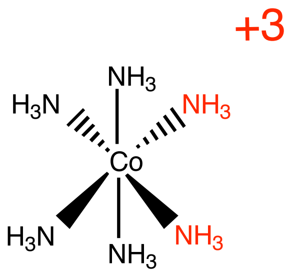
Compete for Mg(II) sites on Many Proteins

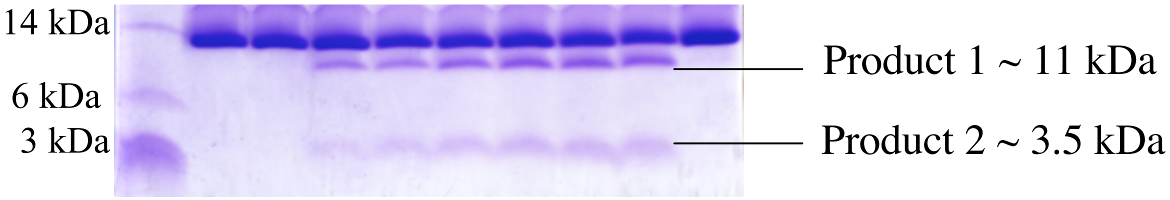


Excitation into LMCT states produce reactive intermediates:

$NH_3^{\cdot +}$ $Cl^{\cdot}$ $Br^{\cdot}$ $OCOO^{\cdot -}$

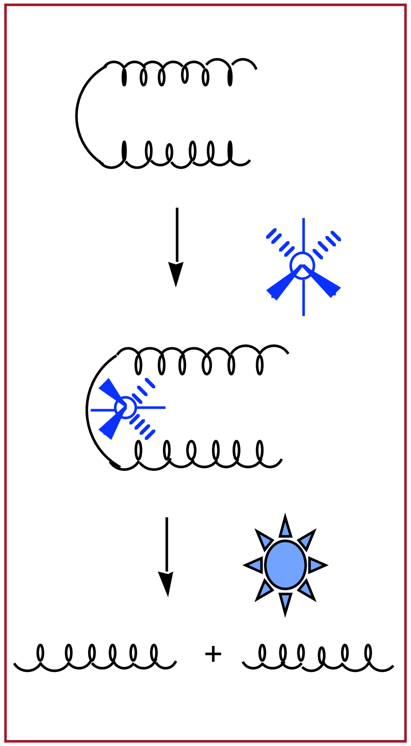
Photocleavage of Lysozyme (75 μM) by CoHA





Lane	2	3	4	5	6	7	8	9	10
CoHA 5 mM	-	+	+	+	+	+	+	+	-
hν Min.	0	0	10	20	30	40	50	60	60

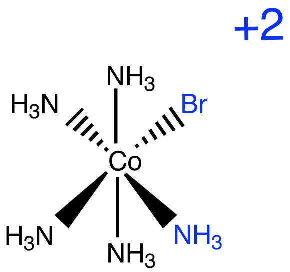
10 % SDSPAGE, 10 mM Tris-HCl, pH 7, Ex. 310 nm

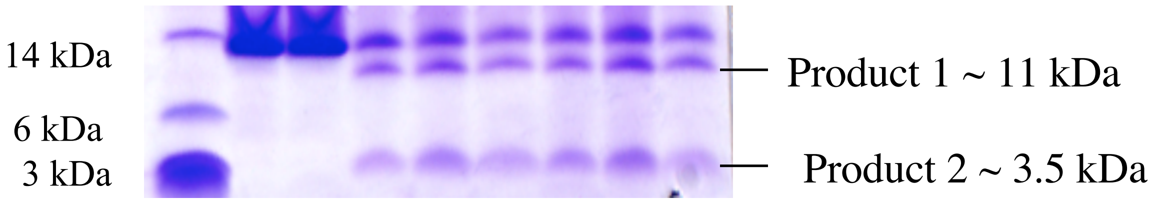


This is the first metal complex to induce Protein Photocleavage

Inorganic Chemistry 2005

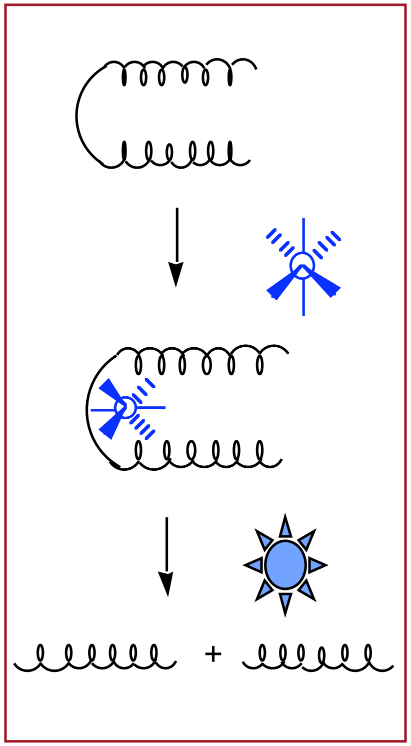
Lysozyme/CoPABr Photoreaction: Irradiation Time Dependence



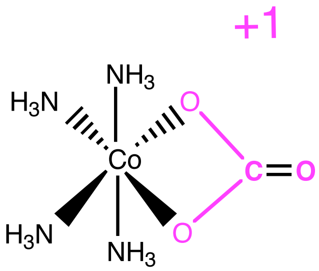


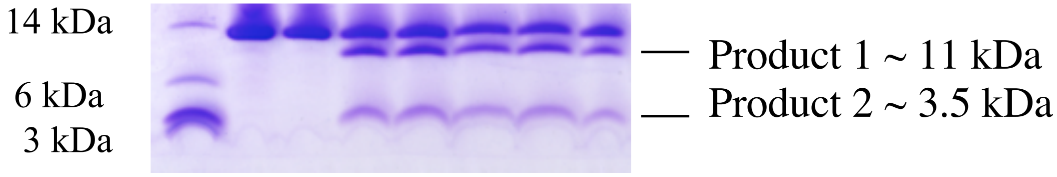
Lane	2	3	4	5	6	7	8	9
CoPABr 2.5 mM	-	+	+	+	+	+	+	+
h*ν* Min.	0	0	10	20	30	40	50	60

10 % SDSPAGE, 10 mM Tris-HCl, pH=7, Ex. 310 nm



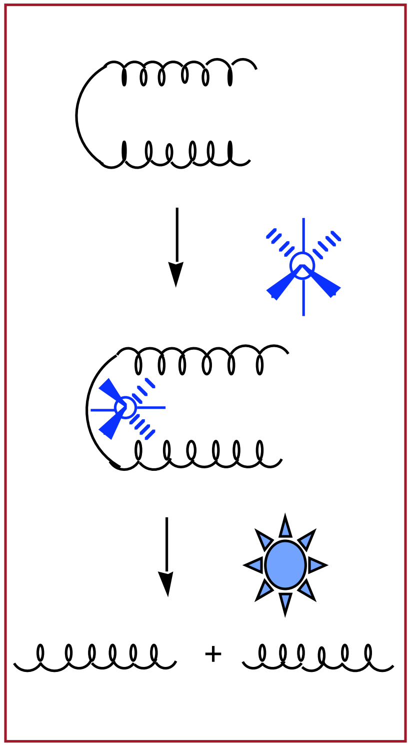
Visible Light Induced Photocleavage of Lysozyme by CoTA





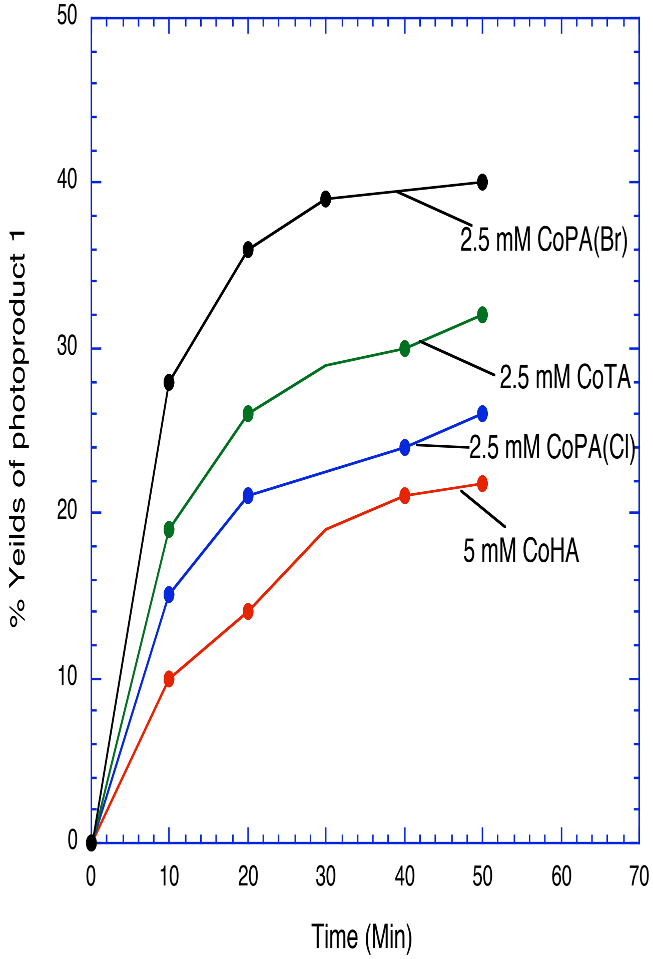
	1	2	3	4	5	6	7	8
CoTA	-	-	+	+	+	+	+	+
h*ν* (nm)	-	-	-	310	330	350	370	**390**

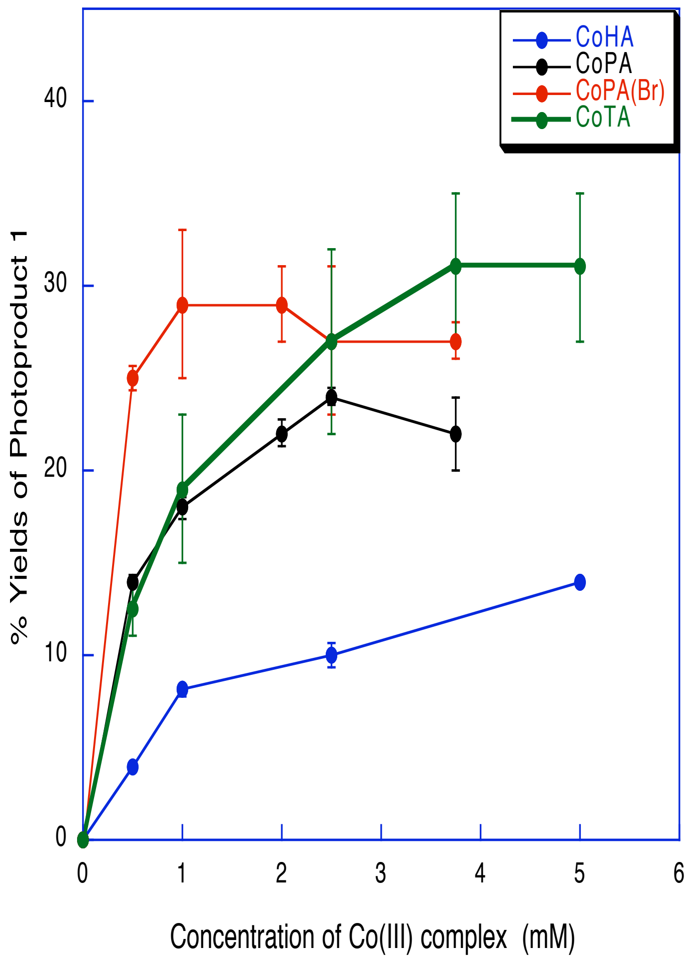
10 % SDSPAGE, 10 mM Tris-HCl, pH=7, Ex. 310-**390** nm

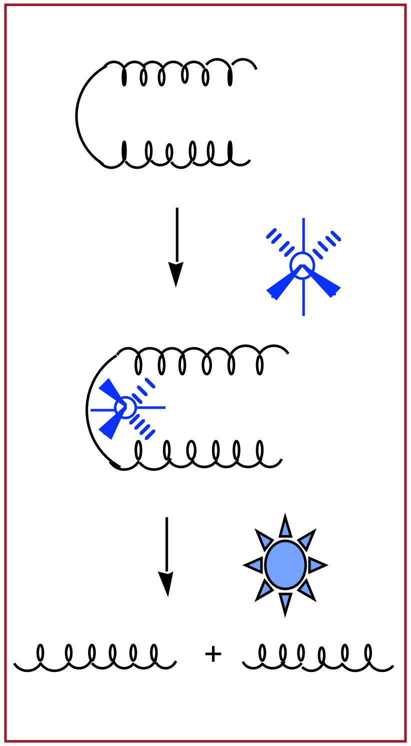


This is the first example of protein photocleavage with visible light!

Photoproduct Yields Increase with Irradiation Time, and Metal Complex Concentration







Lysozyme is cleaved at Lys97-Ile98: Sequencing and Mass Spectra

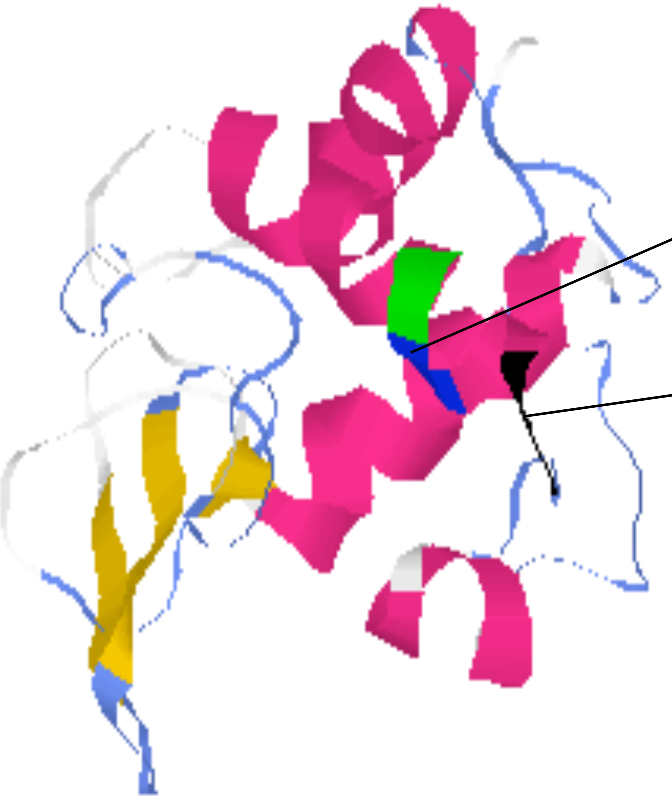
N-terminal peptide, 96 residues　C-terminal peptide, 33 residues

NH_2-Lys-Val-Phe……… Lys(97) - Ile(98)……..Cys-Arg-Leu-COOH

10,643 Da

3,699 Da

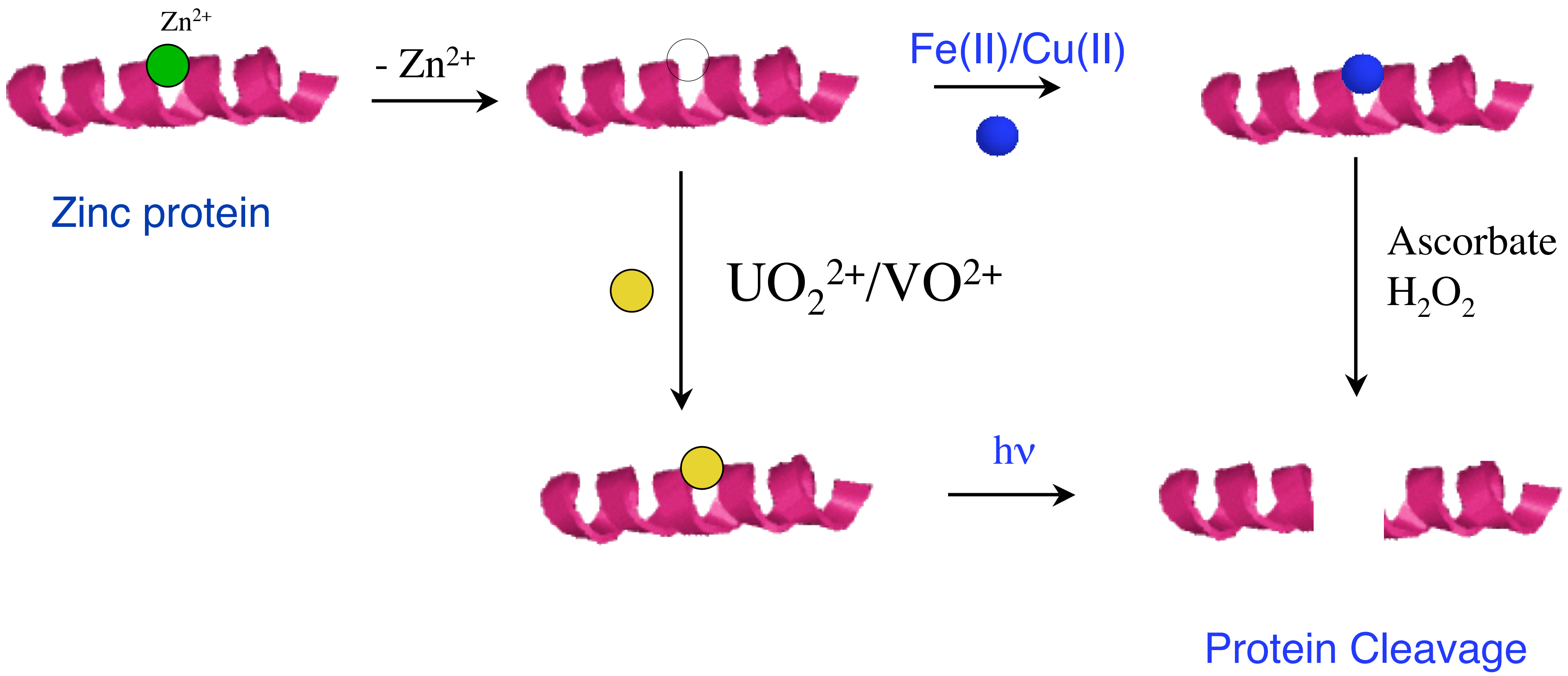
Second Cleavage site ?



Protein Cleavage with Metal Ions: Footprinting Zn sites on Proteins

(Zinc Homeostasis Group)

Strategy



$$\begin{tikzcd} \mathrm{Fe(II)} + H_2O_2 \arrow[r] & \mathrm{Fe(III)} + OH^{\bullet} \arrow[l, bend left, "\text{Ascorbate}"] \end{tikzcd}$$

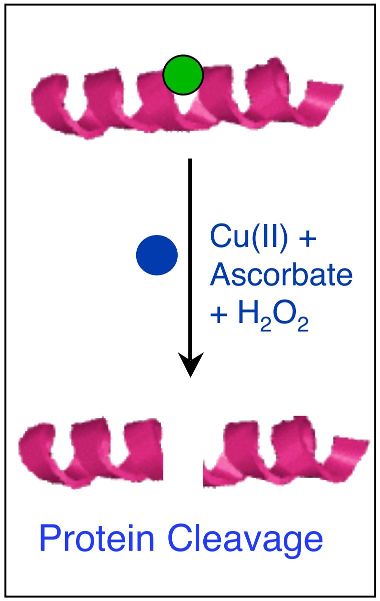
$$\mathrm{Cu(II)} + \text{Ascorbate} \rightarrow \mathrm{Cu(I)} \xrightarrow{H_2O_2} \mathrm{Cu(II)} + OH^{\bullet}$$

Biochem. Biophys. Acta, 1995, *1387*, 309.

Cu(II) induced cleavage of Human Serum Albumin (HSA)

Lanes	1	2	3	4	5	6	7	8	9
15 µM HSA	-	+	+	+	+	+	+	+	+
15 µM $CuSO_4$	-	-	+	+	-	-	+	+	-
500 µM H_2O_2	-	-	+	-	+	-	+	-	+
500 µM Asc	-	-	+	-	-	+	-	+	+

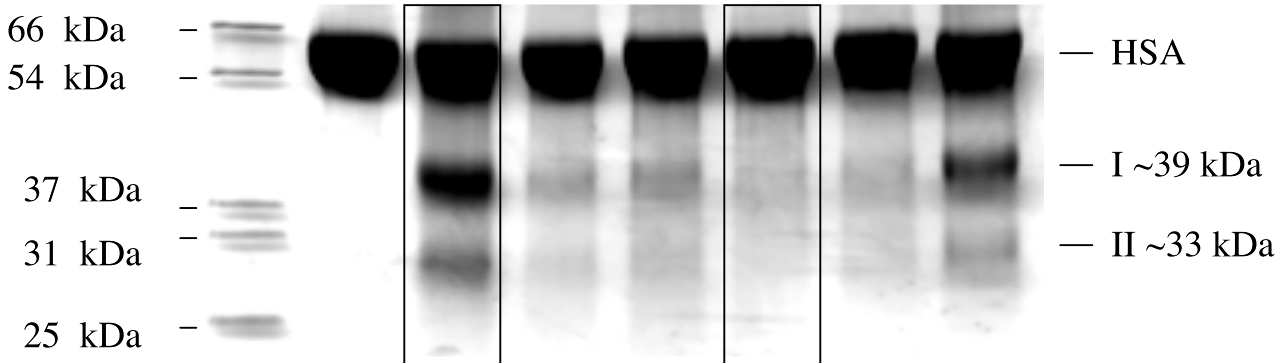
90 min, 10 mM TrisHCl pH 7, 9% SDS-PAGE


Cu(II) + Ascorbate + H_2O_2

Protein Cleavage

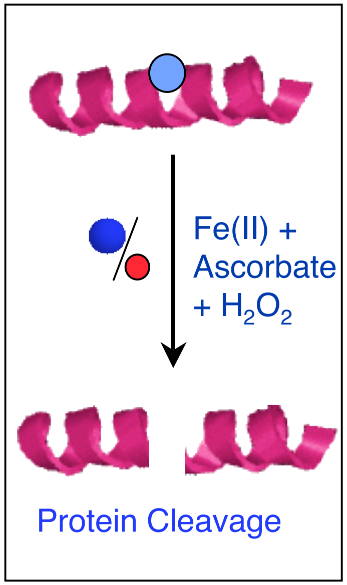
$$Cu(II) + Ascorbate \rightarrow Cu(I) \xrightarrow{H_2O_2} Cu(II) + OH^{\bullet}$$

Fe(II) Cleaves HSA, and Zn^{2+} Inhibits the Reaction



Lanes	1	2	3	4	5	6	7	8
15 μM HSA	-	+	+	+	+	+	+	+
15 μM $FeSO_4$	-	-	+	+	+	+	-	+
[$ZnSO_4$] μM	-	-	-	50	100	500	500	-
1 mM NaCl	-	-	-	-	-	-	-	+
500 μM H_2O_2	-	-	+	+	+	+	+	+
500 μM Asc	-	-	+	+	+	+	+	+

9% SDS-PAGE, 90 min at RT, quenched with 1 mM 2-mercaptoethanol

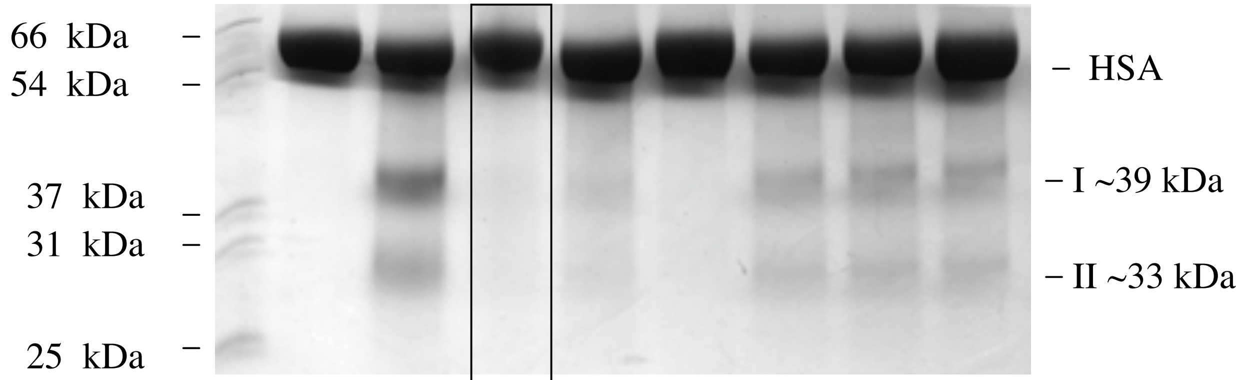


$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\bullet}$$

Ascorbate

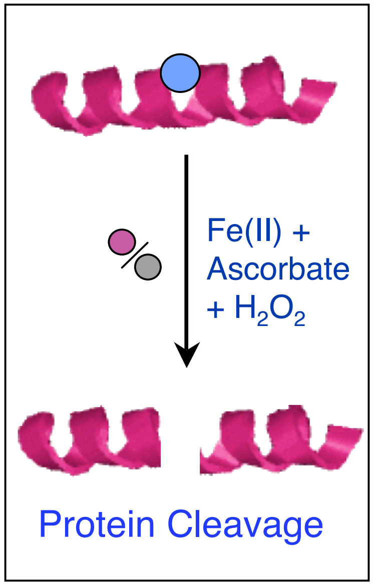
This is the first example of locating Zn site on a protein by footprinting!

Footprinting Metal Sites on HSA with Fe(II)

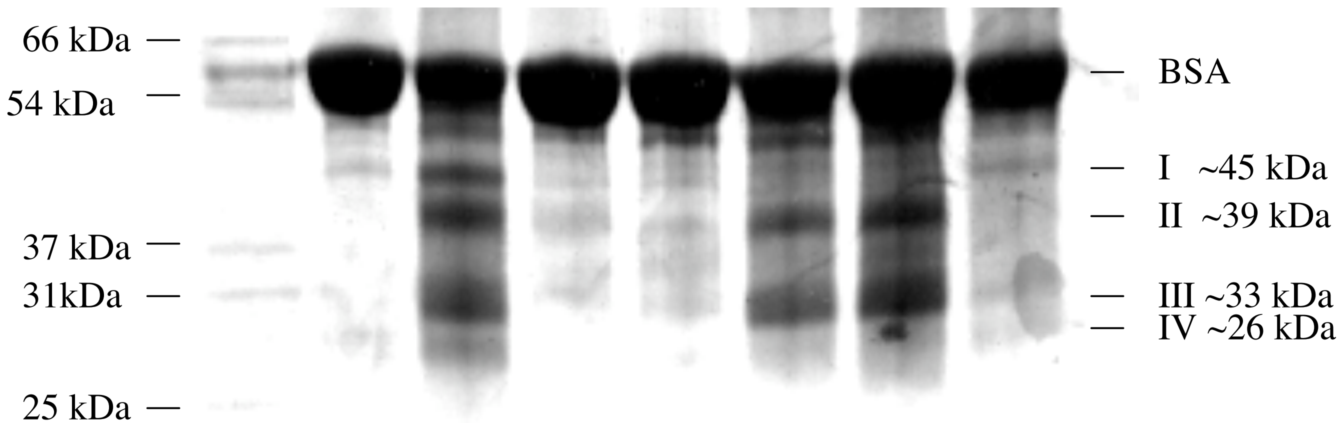


Lane	1	2	3	4	5	6	7	8	9
15 µM HSA	-	+	+	+	+	+	+	+	+
15 µM $FeSO_4$	-	-	-	+	+	+	+	+	+
500 µM M^{n+}	-	-	-	Zn^{2+}	Cd^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Mn^{2+}
500 µM H_2O_2	-	+	+	+	+	+	+	+	+
500 µM Asc	-	+	+	+	+	+	+	+	+
%BSA	-	100	75	100	100	100	89	89	88

90 min RT, 10 mM TrisHCl pH 7, 9% SDS-PAGE

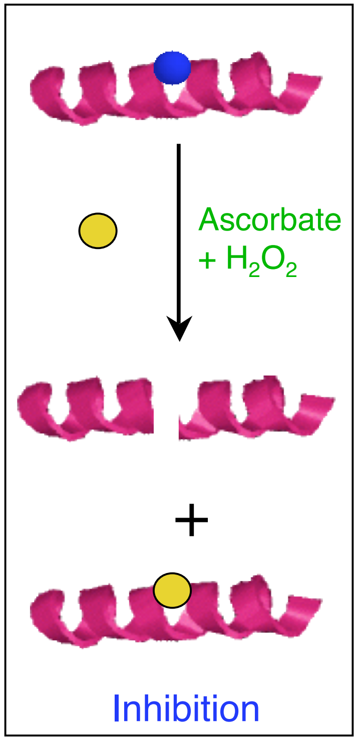


Inhibition of Fe^{2+}-BSA Reaction by Specific Metals



Lanes	1	2	3	4	5	6	7	8
15 µM BSA	-	+	+	+	+	+	+	+
15 µM $FeSO_4$	-	-	+	+	+	+	+	+
1 mM M^{n+}	-	-	-	Cr^{3+}	Al^{3+}	Ce^{3+}	Pb^{2+}	Hg^{2+}
500 µM H_2O_2	-	-	+	+	+	+	+	+
500 µM Asc	-	-	+	+	+	+	+	+
%BSA	-	100	55	94	95	73	71	93

Reacted for 90 min at Room Temp in Water pH 7, 9% SDS-PAGE



Metallomics: Finding Multiple Metal Binding Sites on Proteins

Redox System	Metals that Inhibit (values in percent)												
	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	Cr^{3+}	Mn^{2+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Cd^{2+}	Hg^{2+}	Pb^{2+}	Ce^{3+}
BSA-Cu	-	-	-	-	25	-	40	15	10	-	-	10	-
BSA-Fe	-	-	-	85	85	60	100	85	50	-	85	35	40
HSA-Cu	-	-	-	15	40	-	100	-	25	x	100	30	50
HSA-Fe	-	-	-	ND	100	50	55	55	45	x	-	100	ND

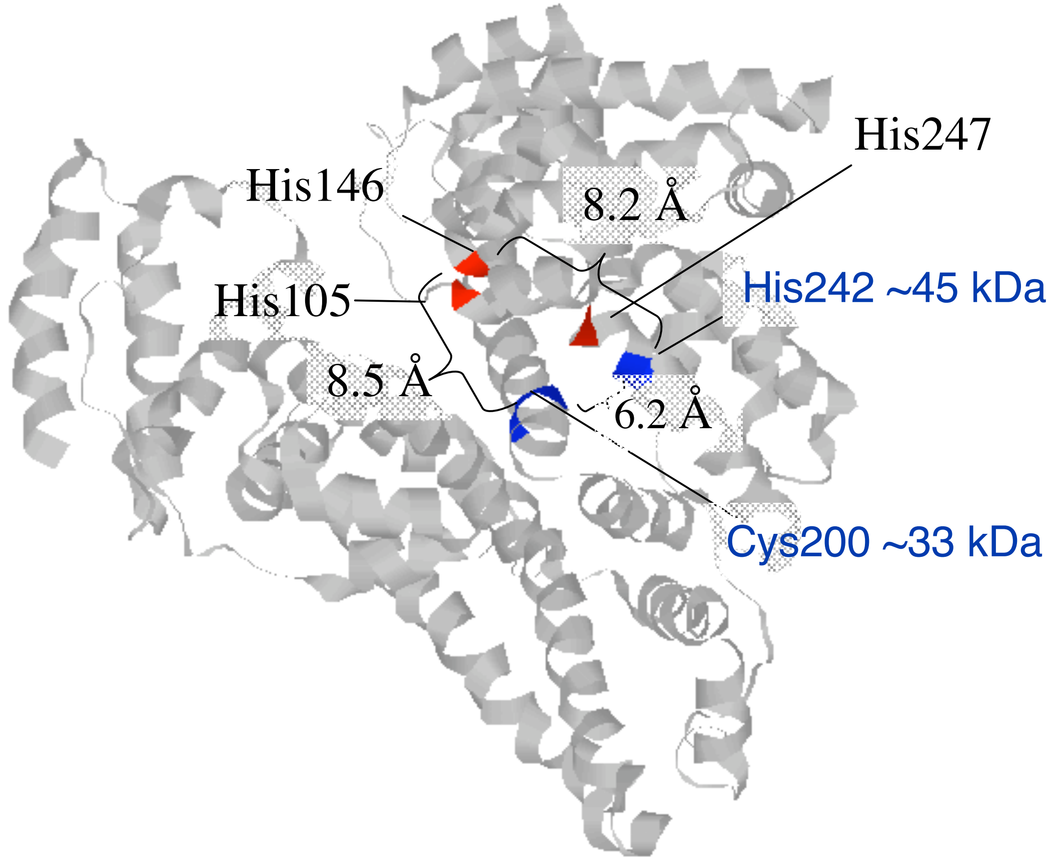
- No inhibition of cleavage; ND Not determined; X To be repeated

Selected Properties of Metals Used

Properties	Metals											
	Al^{3+}	Cr^{3+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Hg^{2+}	Pb^{2+}	Ce^{3+}
Geometry	Oh	Oh	Oh	Oh	Oh	Oh	Oh	Td	Oh	Lin	Oh	Oh
Ligands	O, S	N, O	O, N	O, N, S	N, O	N, O	N, O	N, O, S	S, N, O	S	O	O
E^{o}’ (V)	-1.7 3→0	-.42 3→2	1.5 3→2	.77 3→2	1.9 3→2	1.6 4→2	.16 2→1	-.79 2→0	-.40 2→0	.91 2→1	1.7 4→2	1.7 4→3
Ionic Radii	67 pm	76 pm	81 pm	75 pm	79 pm	83 pm	87 pm	74 pm	109 pm	110 pm	133 pm	115 pm
Abs Max (nm)	< 300	450, 600	~500	> 800	500 - 600	~400	700 - 800	< 300	< 300	< 300	< 300	< 300

Cu(II) 1 Cu(II)-2; Fe(II)-1
1 199 ⇓ 200 242 ⇓ 243 585
H_3N^+-AspAlaHis•••LysCys---AlaSer ••••••ValHis----ThrGlu •••LeuGlyLeu-COOH
(33 kDa) (39 kDa) (45 kDa) (26 kDa)

Proposed Metal Cleavage Sites on HSA

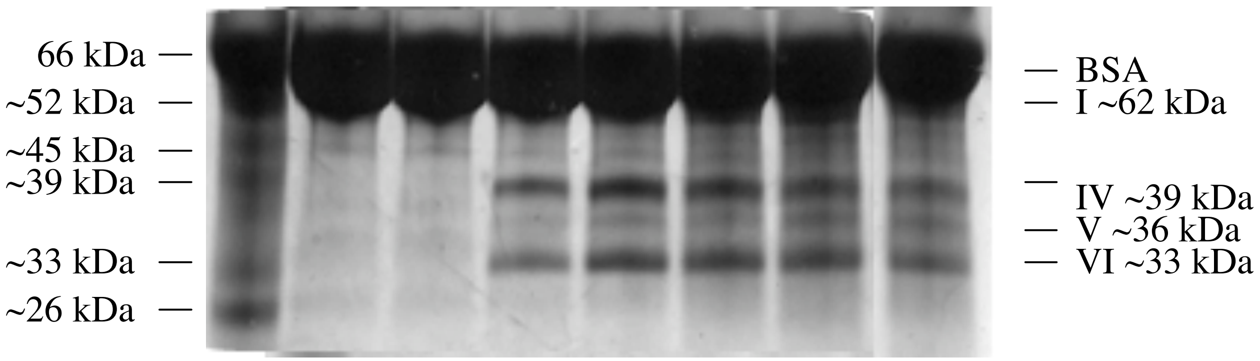


Predicted cleavage site based on estimated masses is between Cys200 and Ala201 for HAS (Gln200 and Lys201 for BSA). This is within 10 Å of the proposed sites.[1,2] The 45, 39, and 33 kDa fragments have the native N-terminus. The 26 kDa fragment was not sequencible.

[1] Sadler, P. J., Viles, J. H. *Inorg. Chem.* **1996**, 35, pp. 4490.

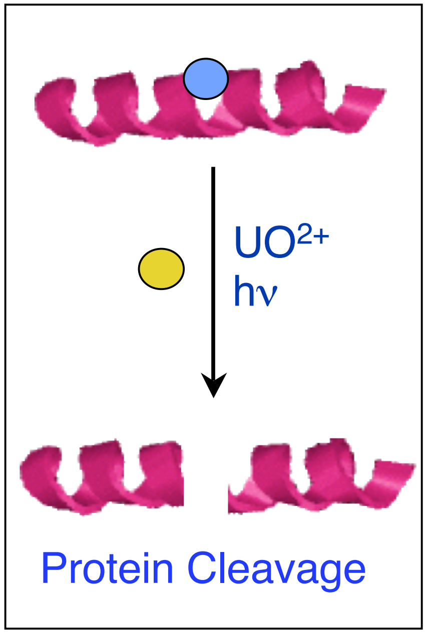
[2] Stewart, A. J.; Blindauer, C. A.; Berenzenko, S.; Sleep, D.; Sadler, P. J. *Proc. Nat. Acad. Sci.* **2003**, *100*, 3701.

PhotoCleavage of BSA by Uranyl



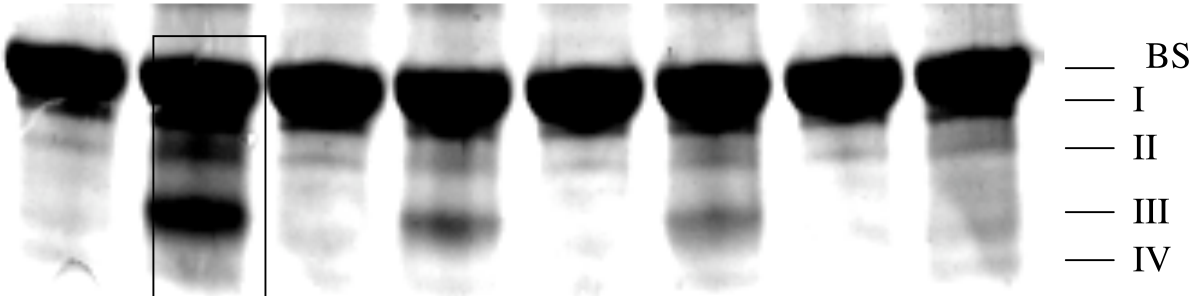
Lanes	1	2	3	4	5	6	7	8
15 μM BSA	-	+	+	+	+	+	+	+
$[UO_2(NO_3)_2]$ μM	-	-	0	25	50	100	150	200
%BSA	-	100	100	87	76	77	67	66

Irradiated at 420 nm for 30 min in DI (pH = 7), 9% SDS-PAGE



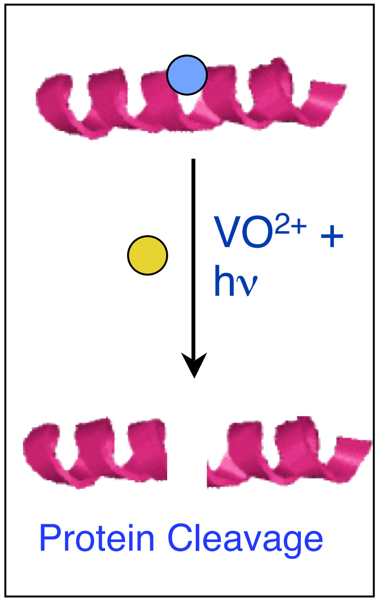
This is the first example of Photocleavage of proteins by Uranyl

PhotoCleavage of BSA by VO^{2+}



Lanes	1	2	3	4	5	6	7	8
15 μM BSA	+	+	+	+	+	+	+	+
200 μM $VOSO_4$	-	+	-	+	-	+	-	+
λ_{irr} (nm)	320	320	330	330	340	340	350	360
%BSA	87	64	87	77	89	77	91	85

Irradiated for 1 hour in DI, 310 nm filter, 9% SDS-PAGE



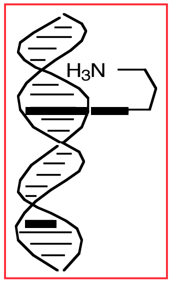
This is the first example of photocleavage of proteins by Vanadyl

DNA-Drug Interactions

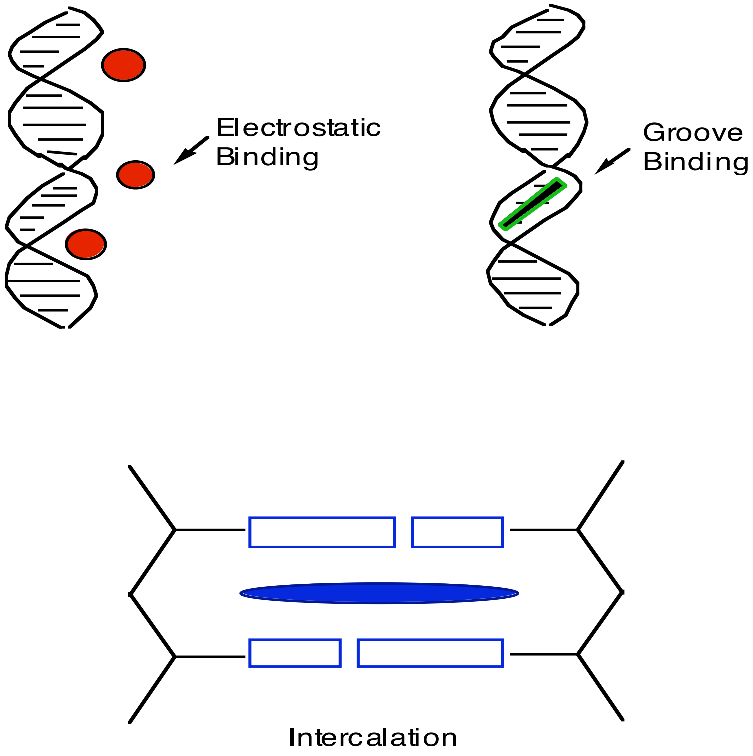
Goal

Quantify drug-DNA Interactions

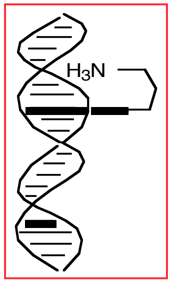
Approach

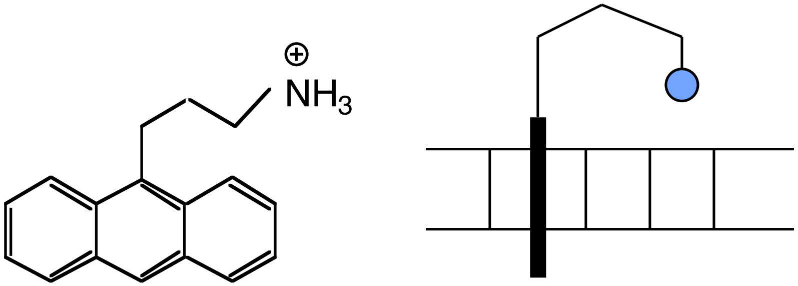


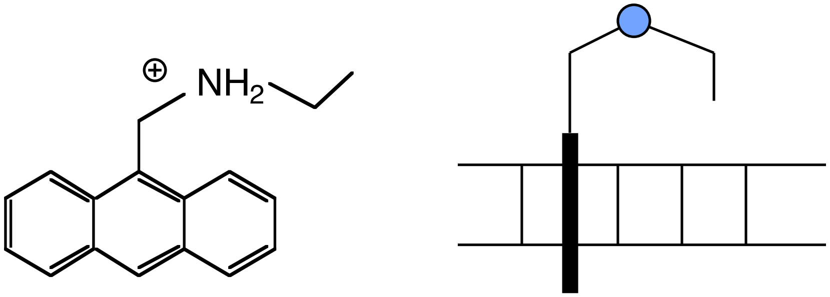
Use Spectroscopy/Calorimetry/Photochemistry



H-Bonding & Sequence Recognition







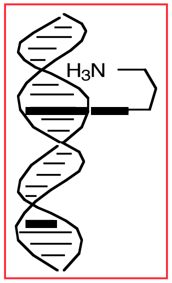
Both bind with affinities greater than AMAC.

Propyl derivative shows selectivity for AT sequences

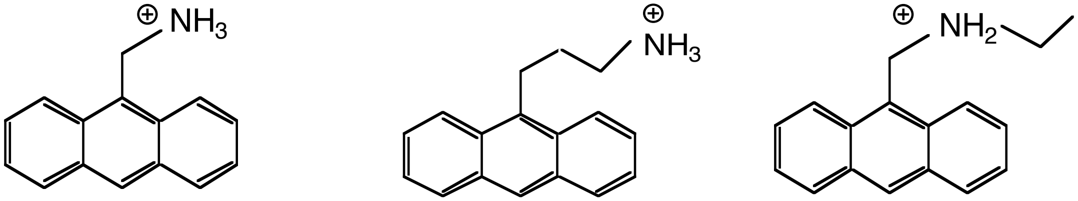
N-Et derivate has no selectivity

Both photocleave DNA

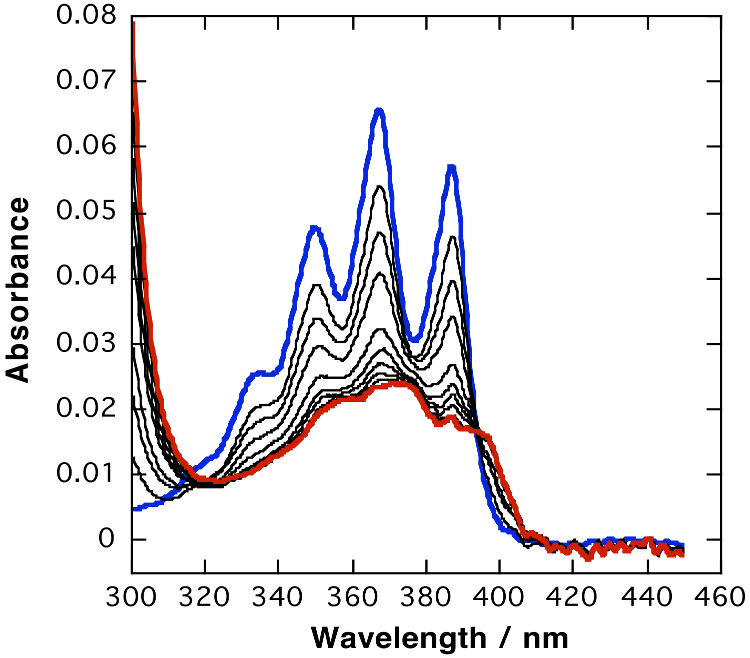
Kumar and Tan, Tetrahedron 2000



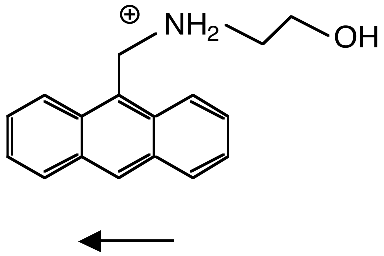
Sequence Selectivity

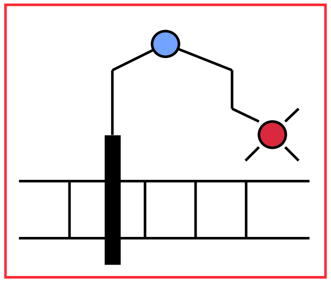


DNA	AMAC (3220) (M^{-1})	APAC (7830) (M^{-1})	N-Et-AMAC (4950) (M^{-1})
Poly (dA-dT)	$2.5\pm0.3x10^{4}$	$3.0\pm0.6x10^{4}$	$2.9\pm0.4x10^{4}$
Poly (dA)-Poly(dT)	-------------------	$1.1\pm0.2x10^{4}$	-------------------
Poly (dG-dC)	$1.5\pm0.1x10^{4}$	$8.9\pm0.2x10^{3}$	$2.7\pm0.2x10^{4}$
Poly (dI-dC)	$2.8\pm 0.1x10^{4}$	$2.4\pm0.1x10^{4}$	$2.4\pm0.3x10^{4}$

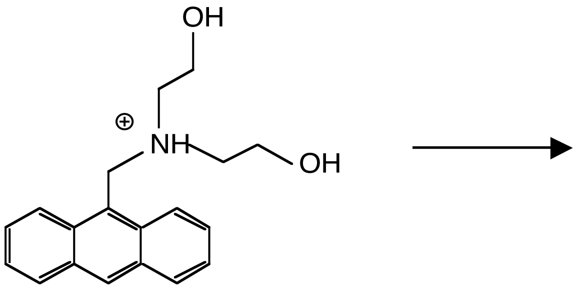


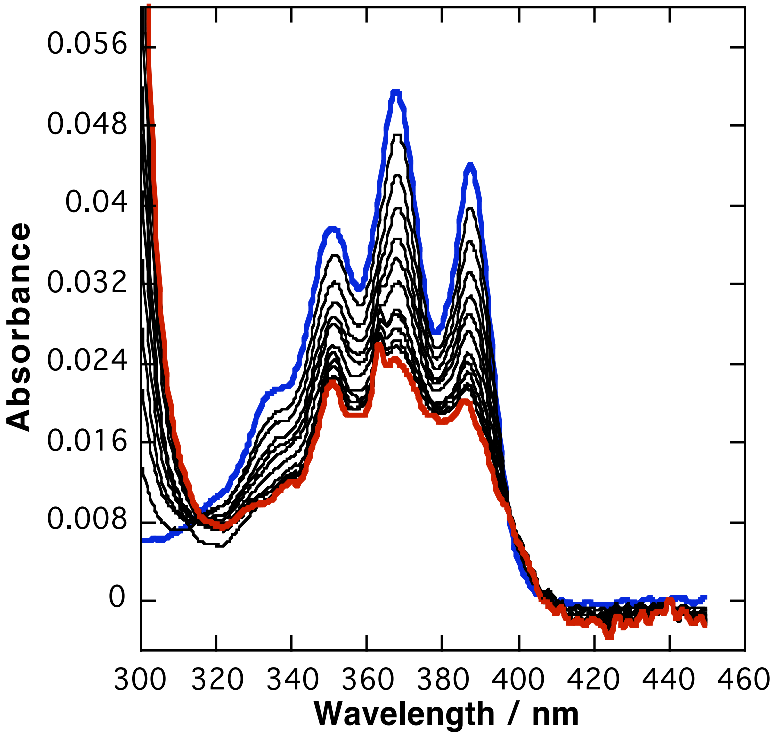
MEA(5μM)/CTDNA (0-146μM)



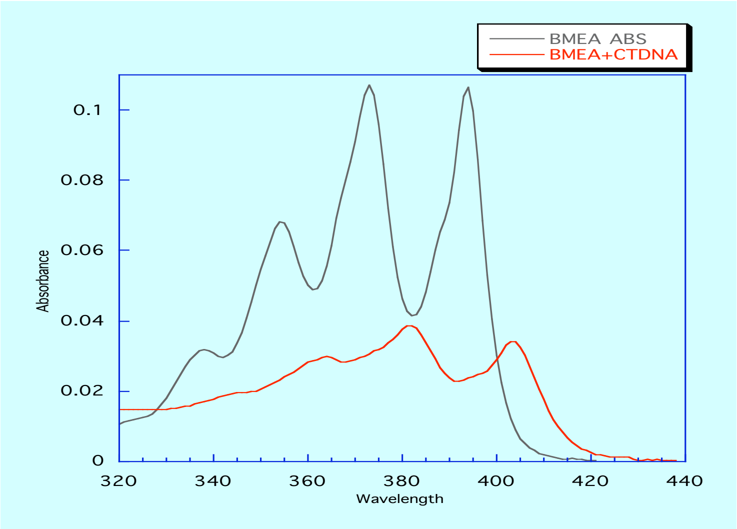


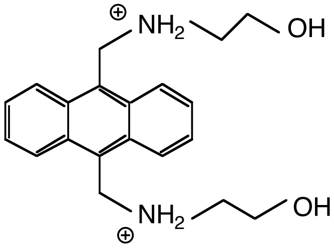
DEA(4μM)/CTDNA (0-174μM)

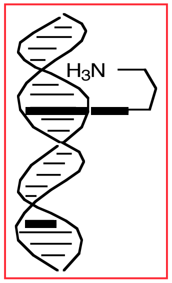




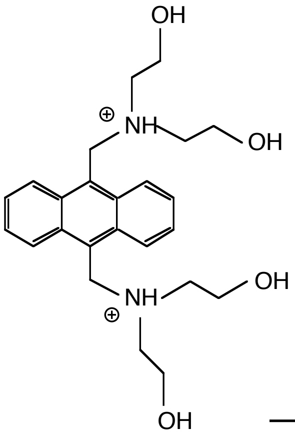
BMEA(12μM)/CTDNA (0-132μM)

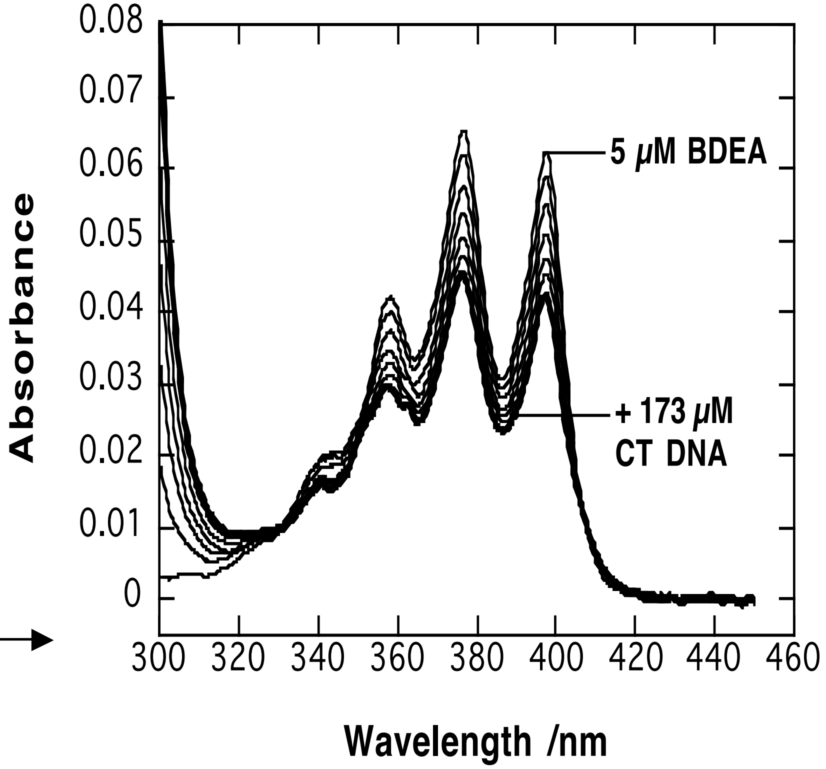




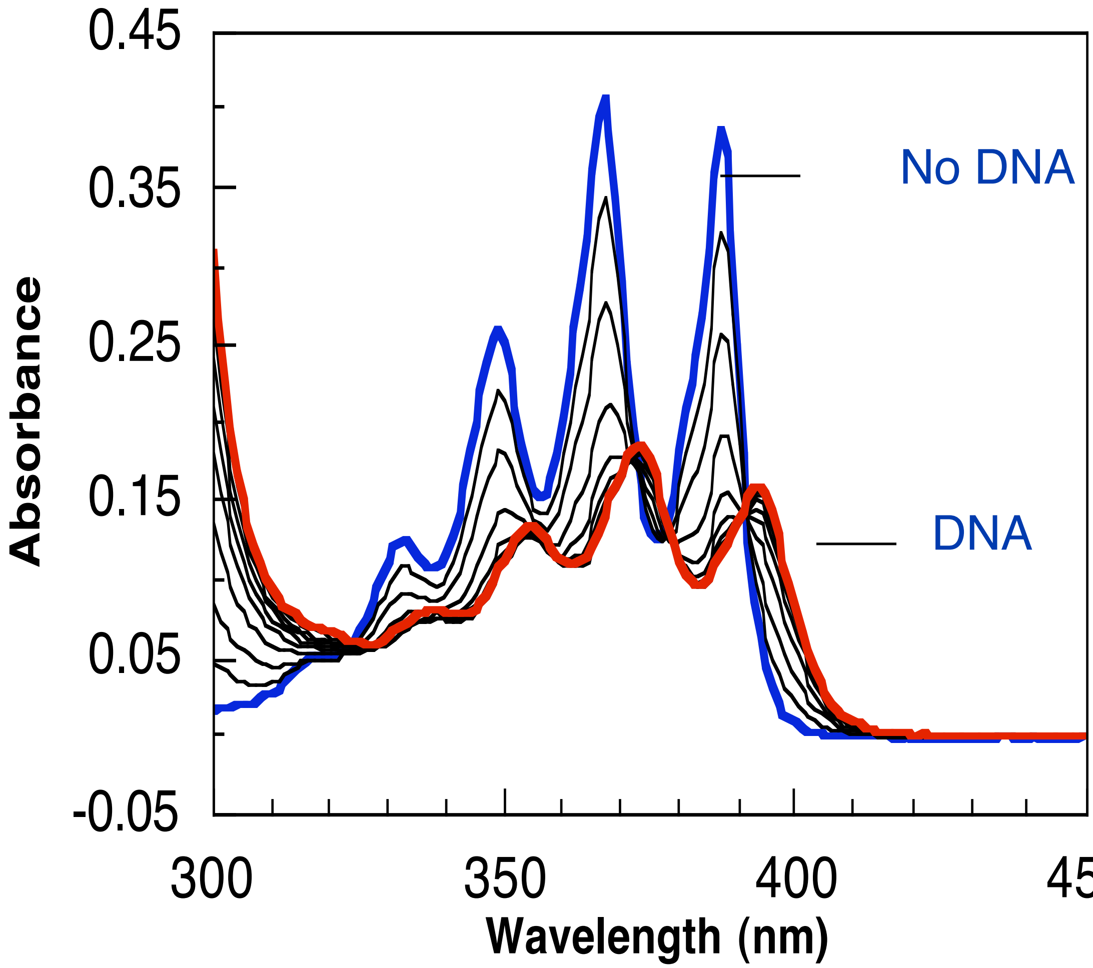


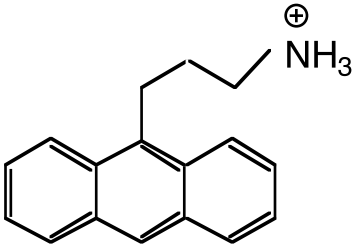
BDEA(5μM)/CTDNA (0-173μM)



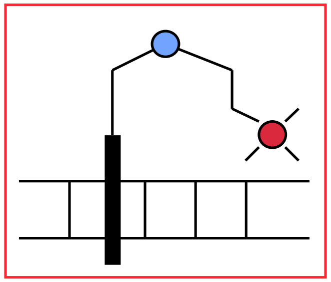


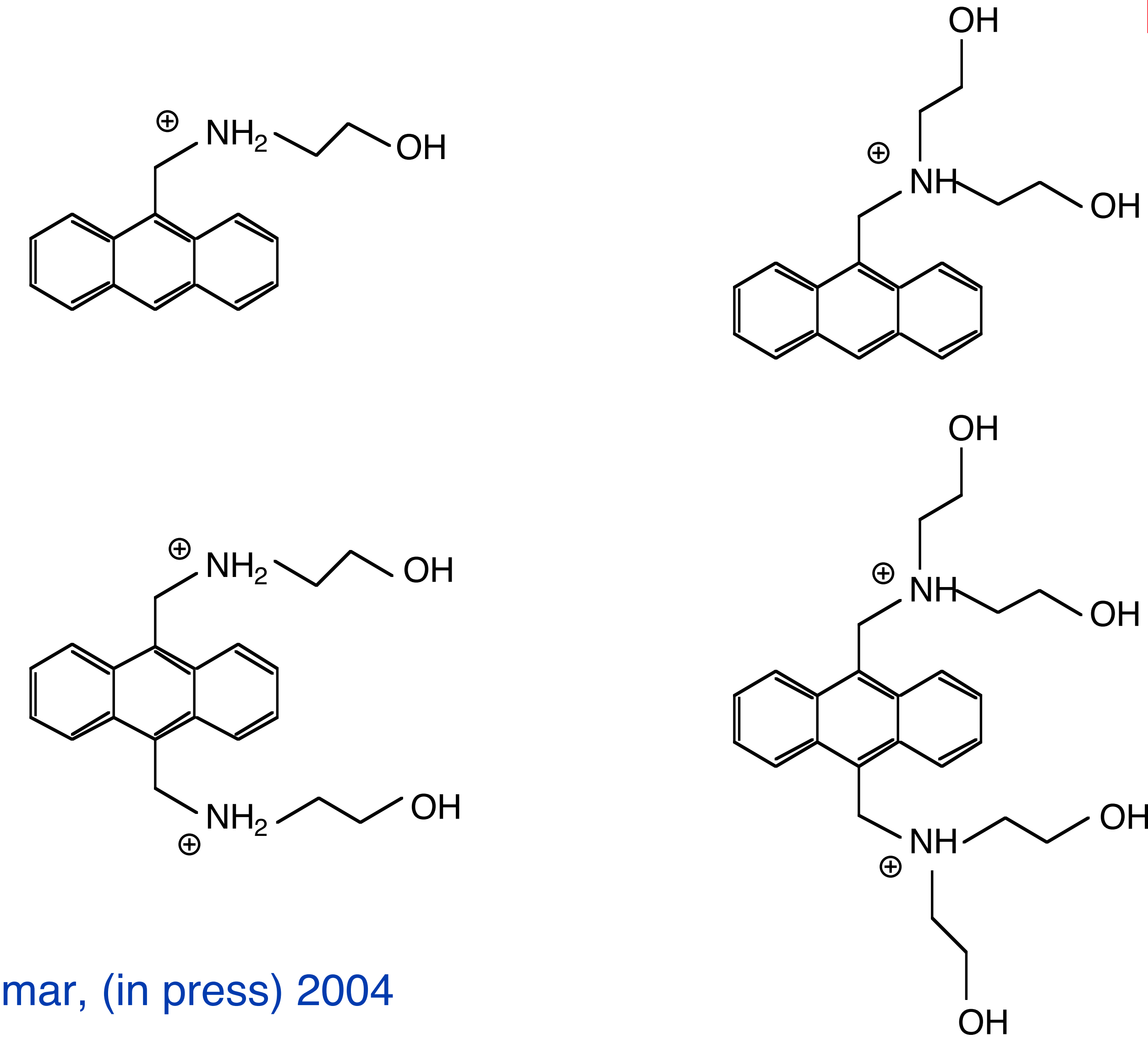
Absorption Titration/APAC (9 μM)/Calf Thymus DNA (0 - 420 μM)





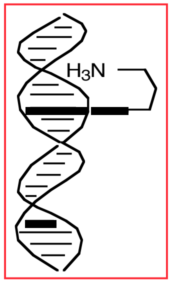
Multiple H-Bonding for Seq. Recognition

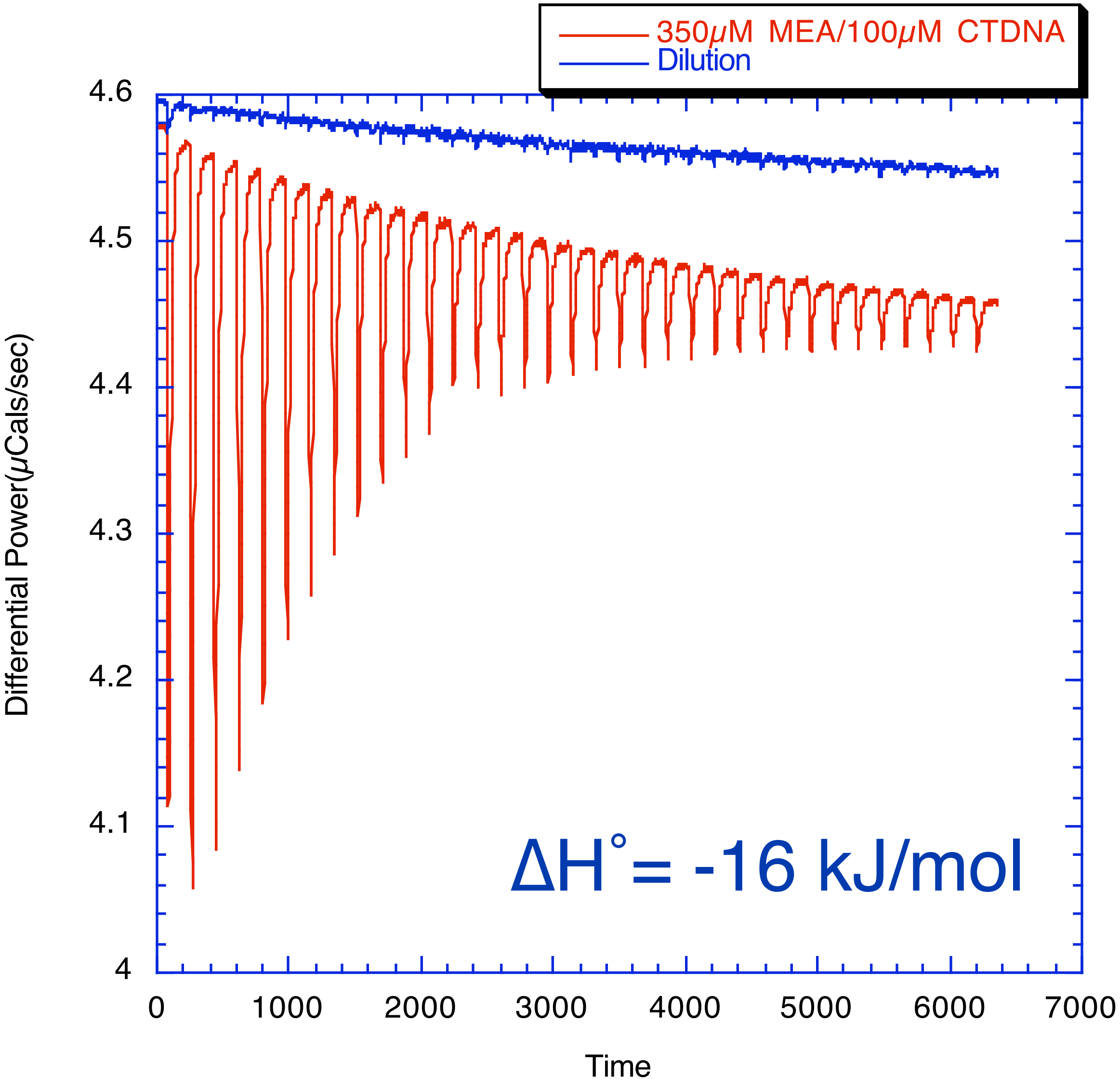




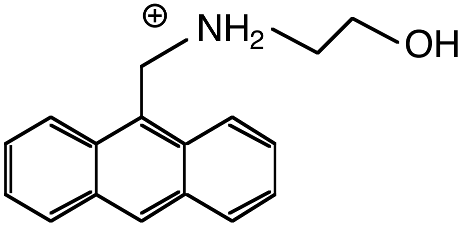
Tan and Kumar, (in press) 2004

Microcalorimetry of MEA + CT DNA (25˚C)

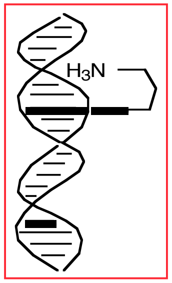


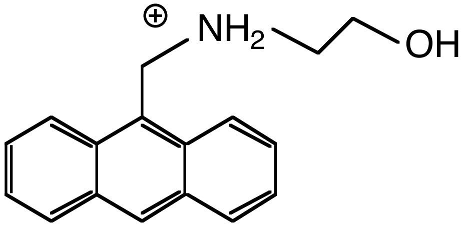


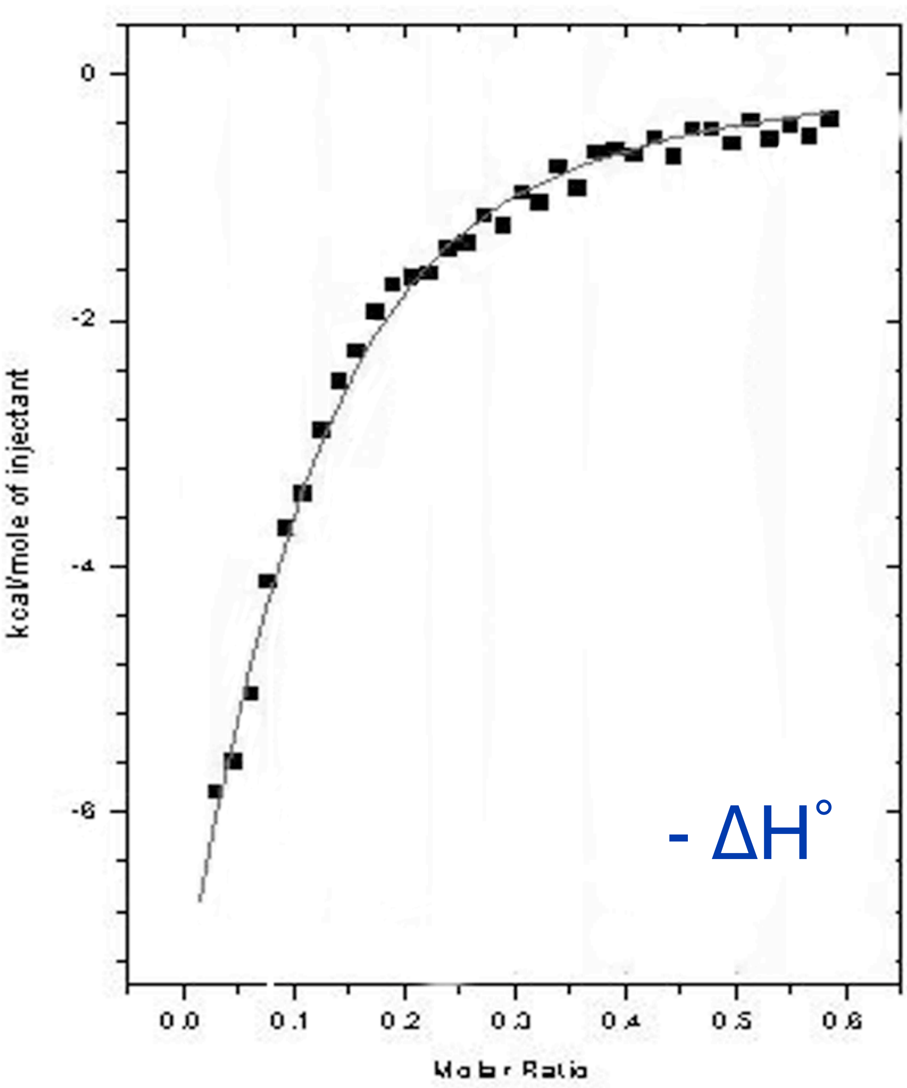
ΔH˚= -16 kJ/mol



Thermodynamics of MEA-CTDNA Interaction



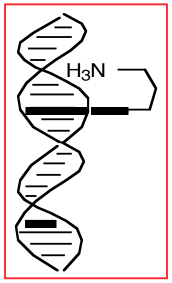


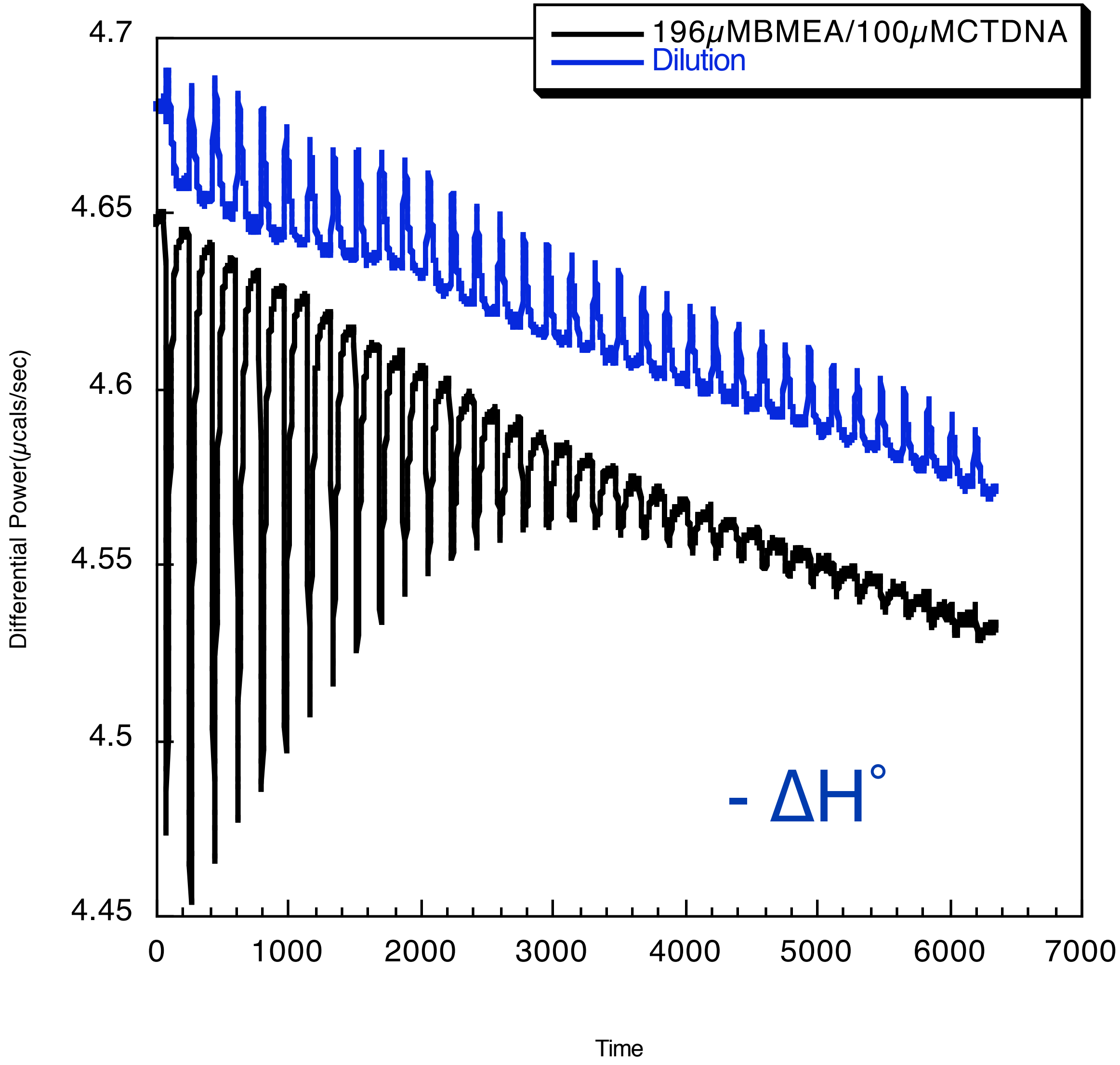


- ΔH°

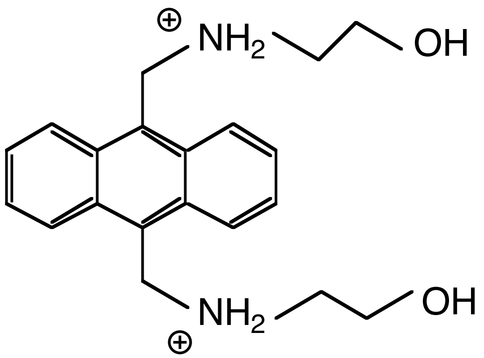
The data was fitted to
“One set of binding sites”
n=0.0788 +/- 0.00409
K= $1*10^5$ +/- 0 M^{-1}
ΔH= $-1.6*10^4$ +/- 768.2 J/mol
ΔS= -31.04 e.u.

Binding of BMEA to CT DNA (25˚C)

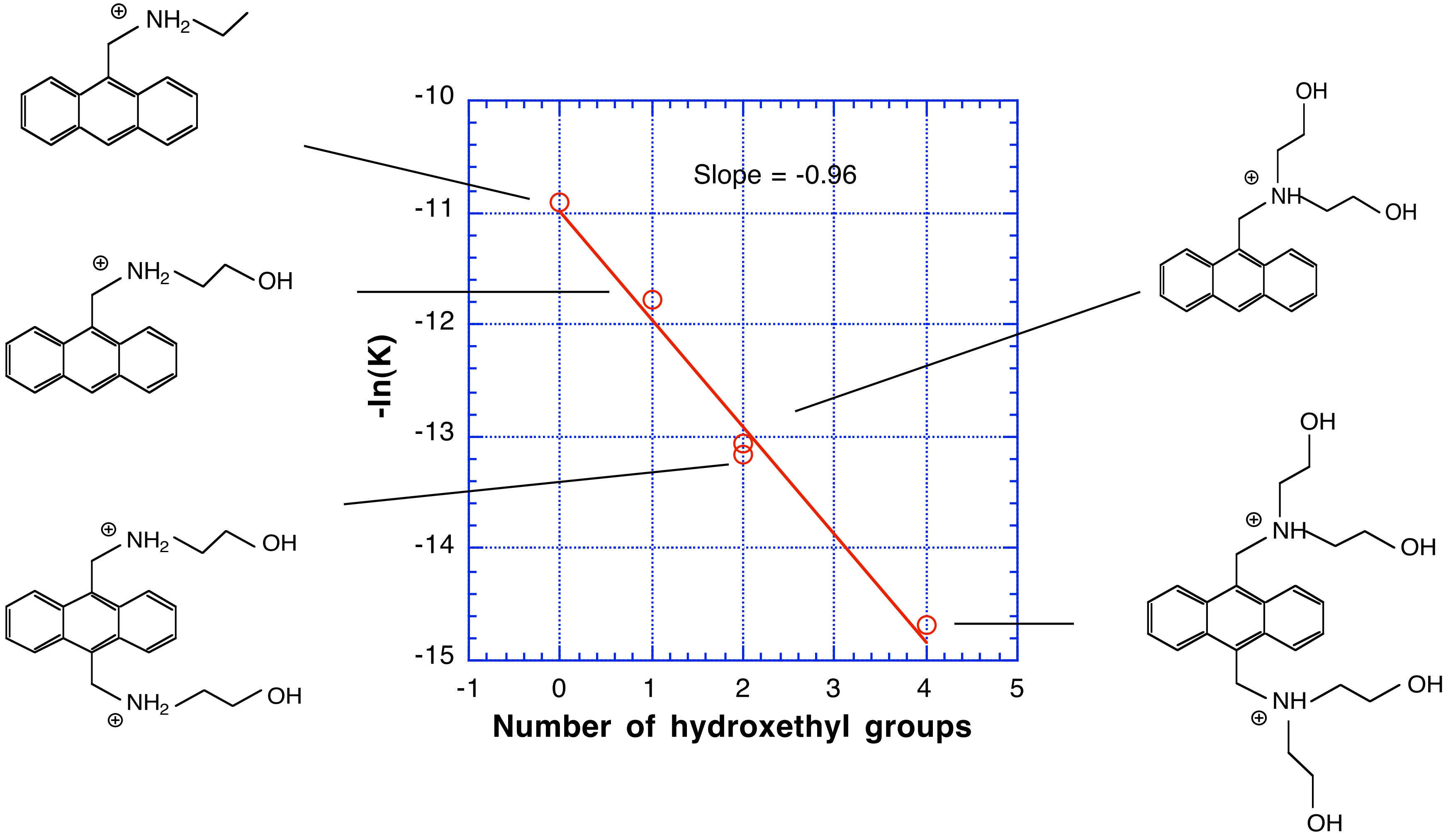




- ΔH°

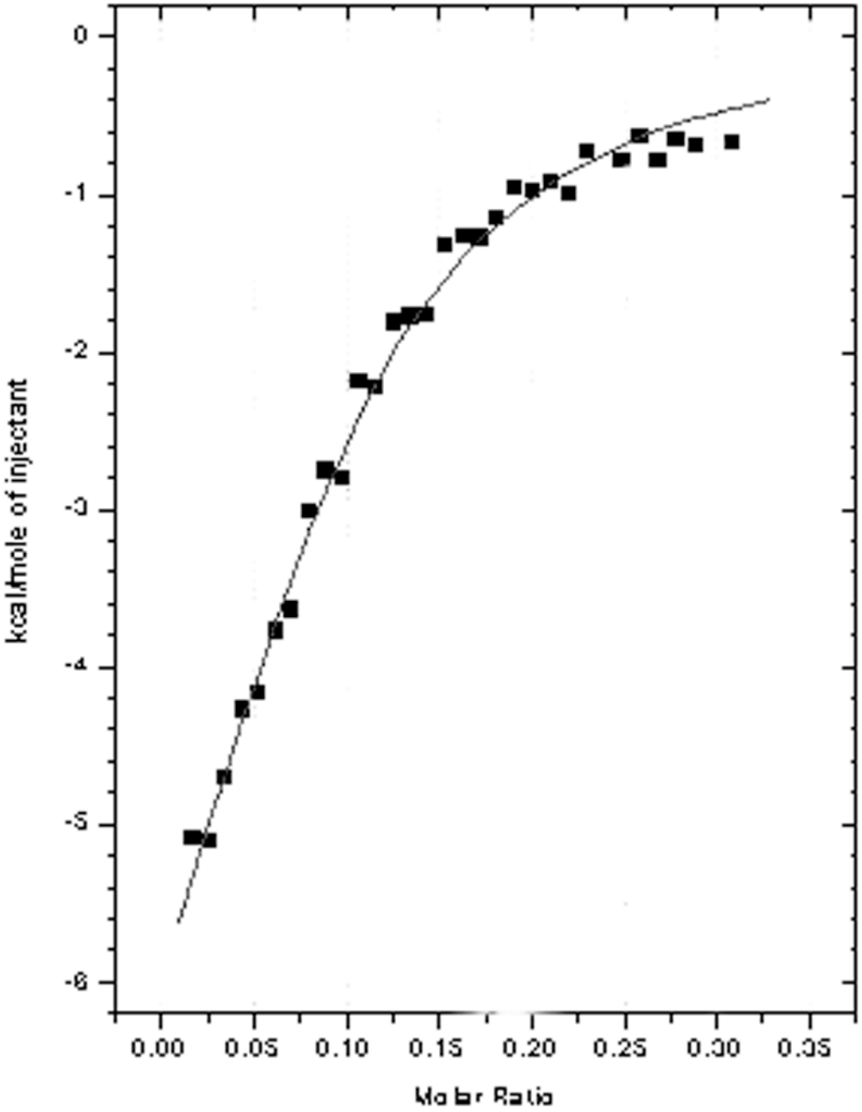


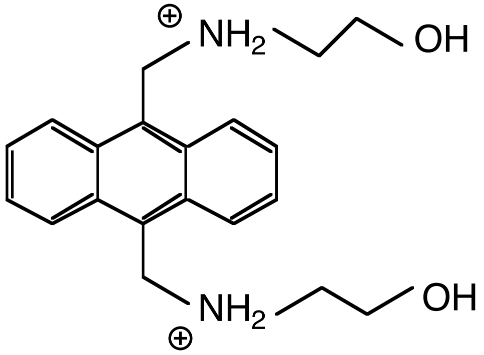
Binding Affinity Increases with the # of H-bonding Groups



Each H-bond contributes 0.75 kcal/mol

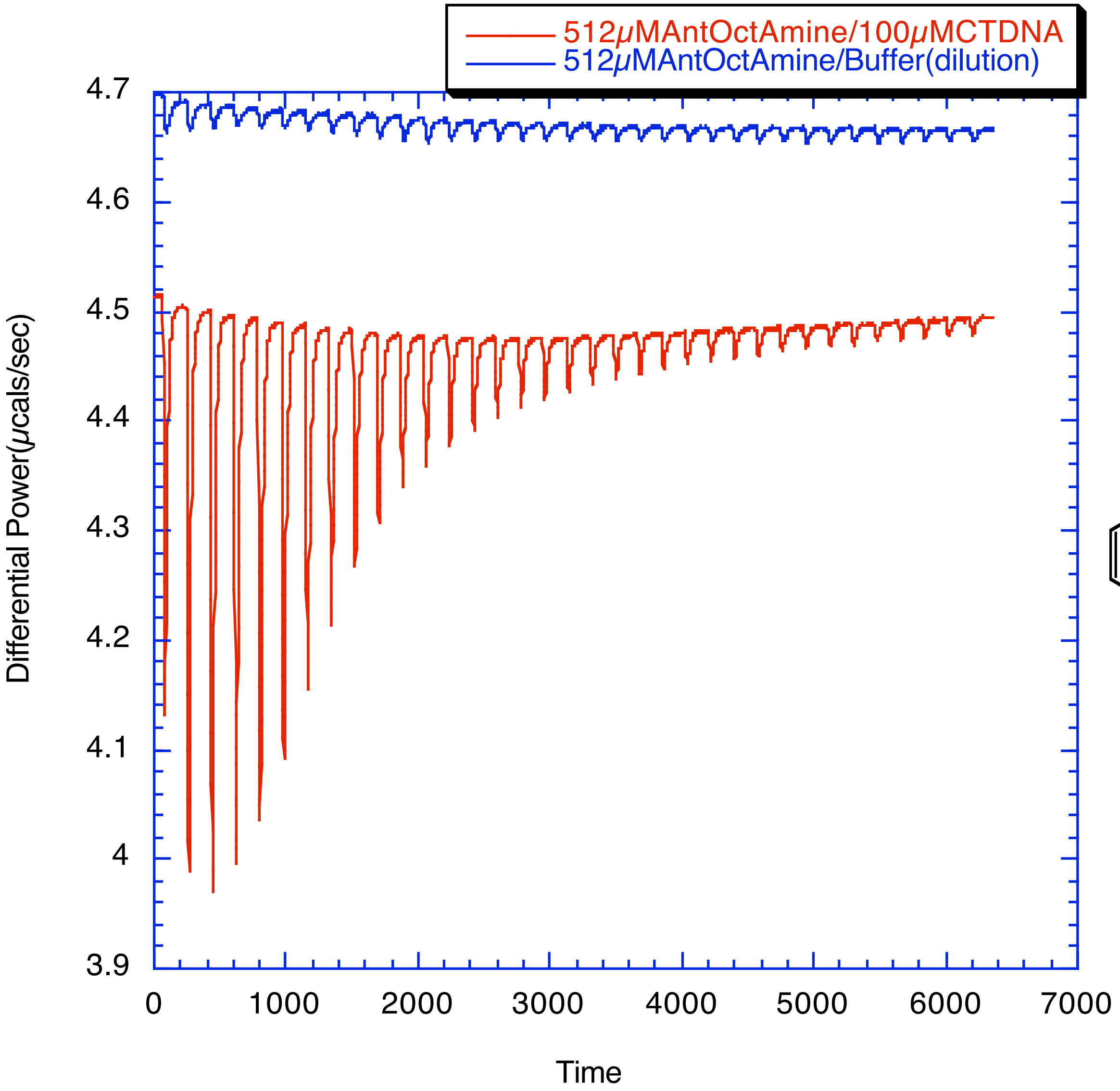
BMEA-CTDNA

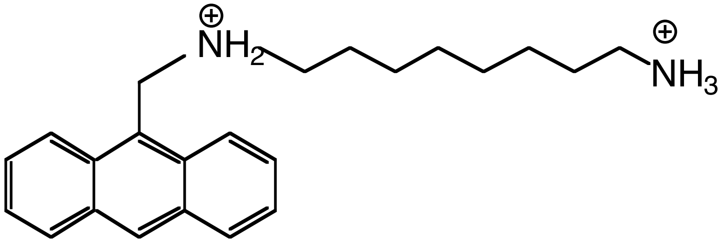




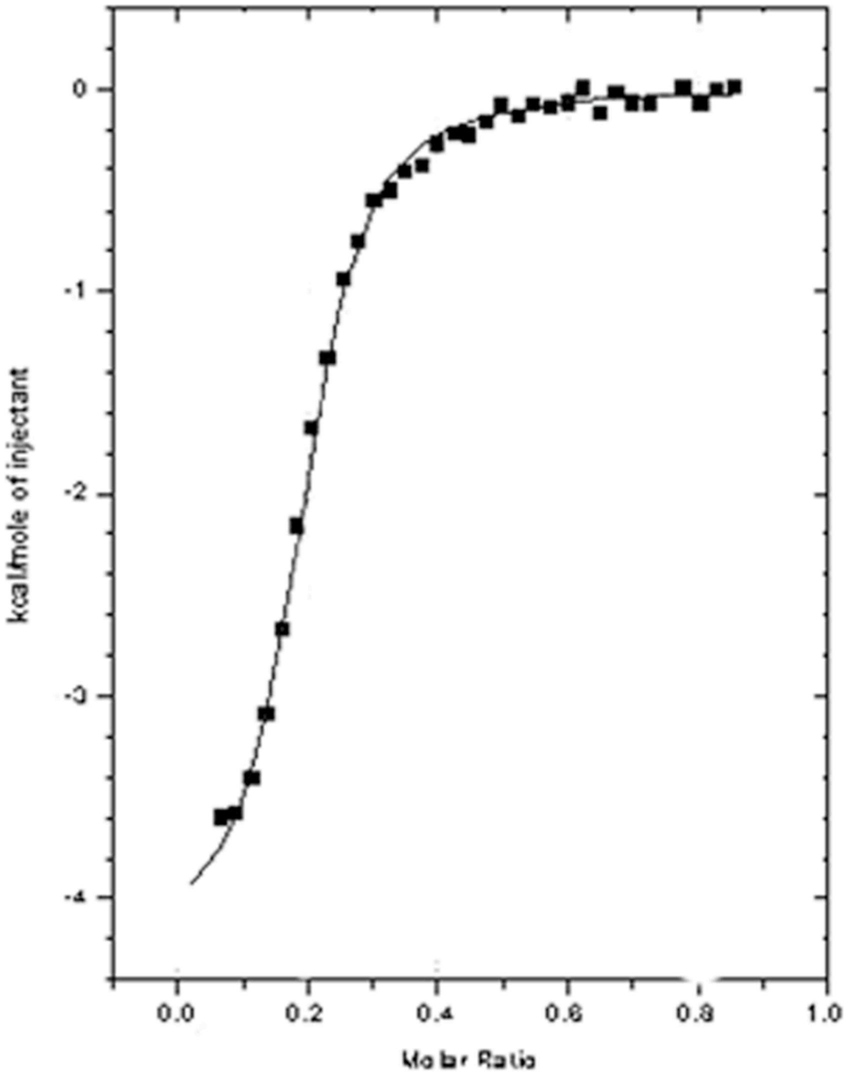
The data was fitted to
“One set of binding sites”
$n=0.08$ +/- 0.002866
$K= 1.9*10^5$ +/- $0.1\ M^{-1}$
$\Delta H= -9357$ +/- 272.7 J/mol
$\Delta S= -7.23$ e.u.

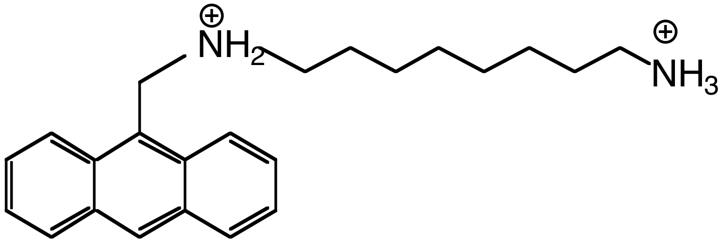
Binding of AODA to CT DNA (25˚C)





Anthraceneoctyldiamine-CTDNA Titrations

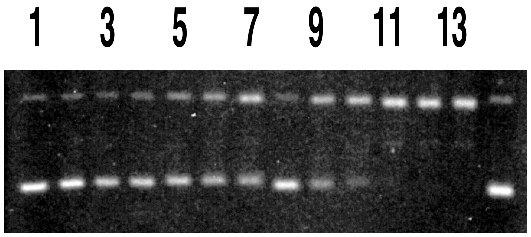


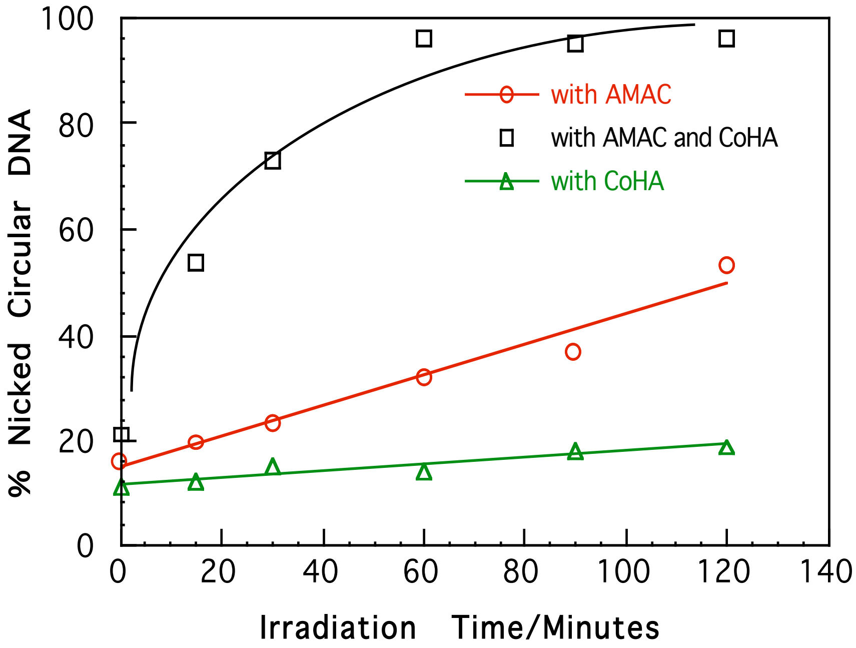


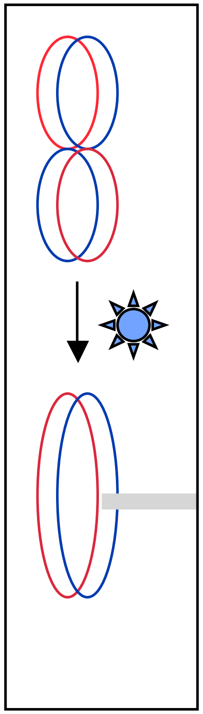
The data was fitted to
“One set of binding sites”
n=0.2 +/- 0.001679
K= $7*10^5$ +/- 0.1 M^{-1}
ΔH= -4241 +/- 39.95 J/mol
ΔS= 12.52 e.u.

DNA Photodamage by AMAC

pUC18 (10 μM), AMAC (50 μM), CoHA (200 μM), Ex 390 nm, 0-120 min



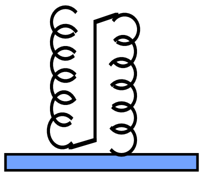




Enzyme-Inorganic Materials

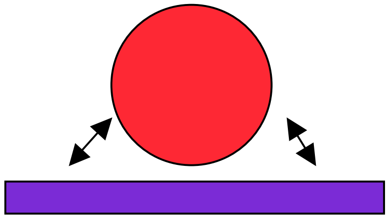
Challa V. Kumar
University of Connecticut
http://jasmin.chem.uconn.edu

Outline



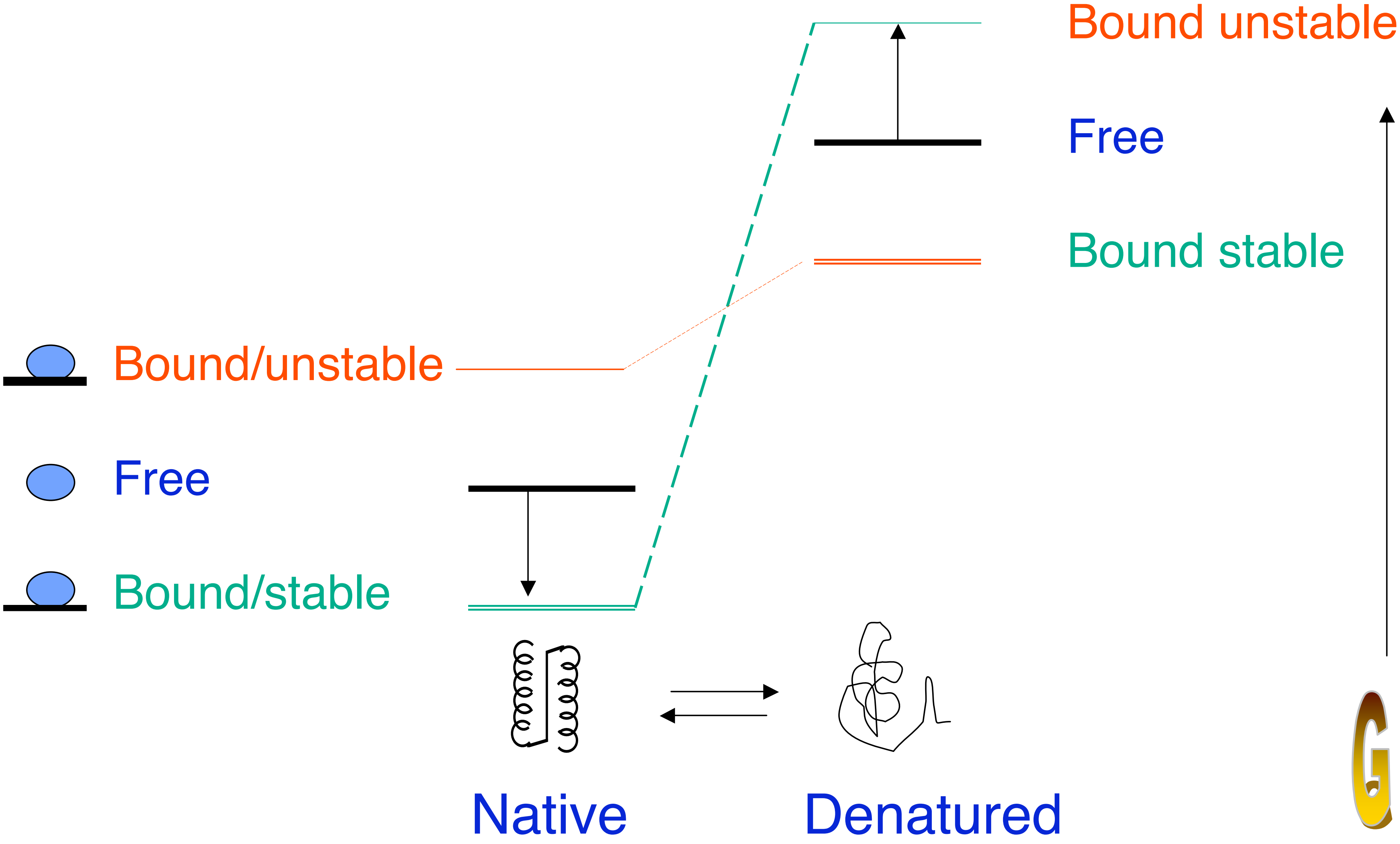
1. Motivation
2. Preparation of Protein-Inorganic Materials
3. Characterization
4. Reversible Thermal Denaturation
5. High Temperature Activity
6. High Temperature Protein Refolding

Motivation



How to Control Protein- Solid Interactions?

How to Improve Enzyme Stability?



Bound unstable

Free

Bound stable

Bound/unstable

Free

Bound/stable

Native

Denatured

G

Solids Used for Protein Binding

1. Organic polymers

2. Silica/Glass

3. Oxides

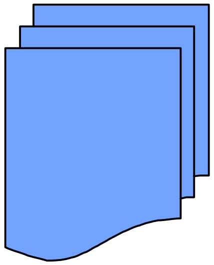
But - most of these are:

Chemically, topologically, and morphologically heterogeneous.

Data interpretation is complex/impossible.

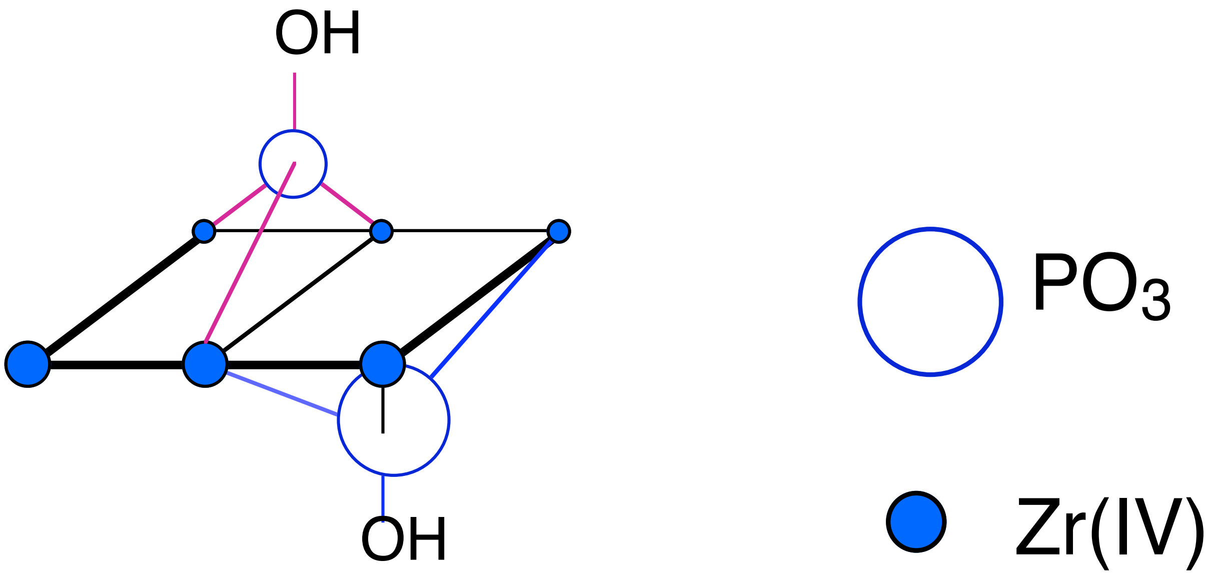
(layered α-$Zr(HPO_4)_2$ is a good alternative)

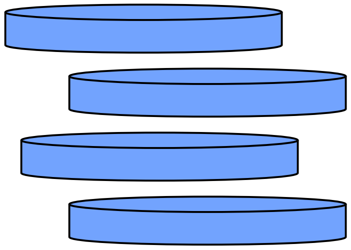
Structure of α-ZrP/ZrRP



$ZrOCl_3 + PO(OH)_3 \text{ --> } Zr(PO_3OH)_2.nH_2O$

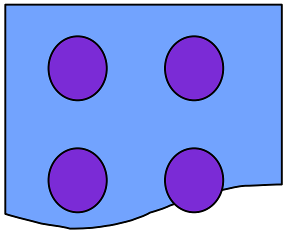
$ZrOCl_3 + RPO(OH)_2 \text{ --> } Zr(PO_3R)_2.nH_2O$

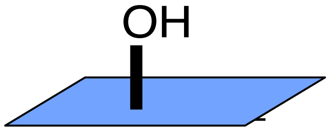




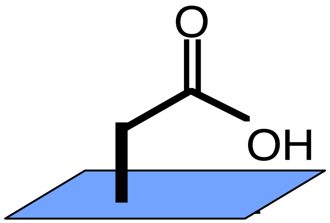
Layered Crystalline Material --- First Reported by Clearfield e

Zr(IV) Phosphonates have Well-defined Surfaces

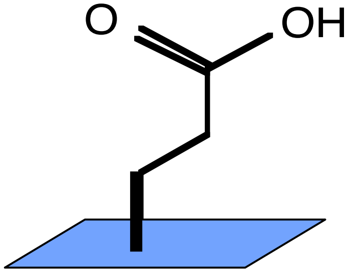




α-ZrP



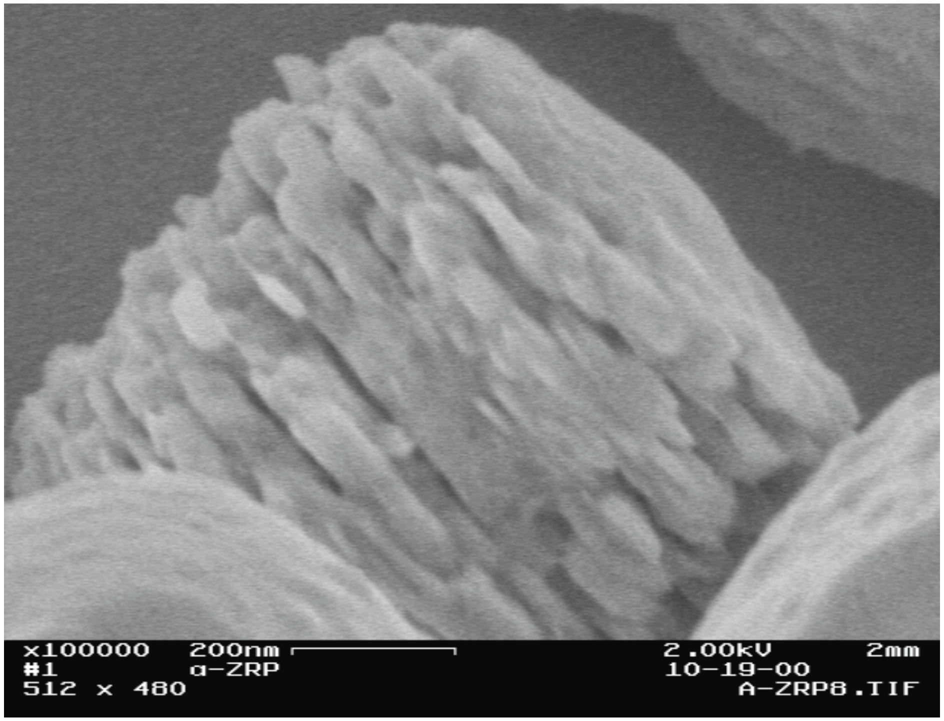
α-ZrCMP



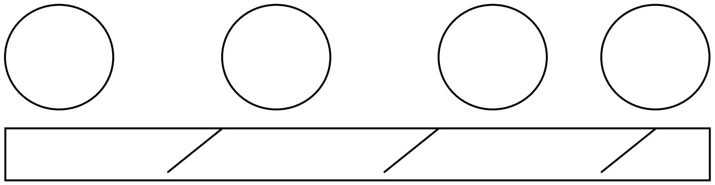
α-ZrCEP

Surface area ~ 5Å x 5Å per phosphate/phosphonate

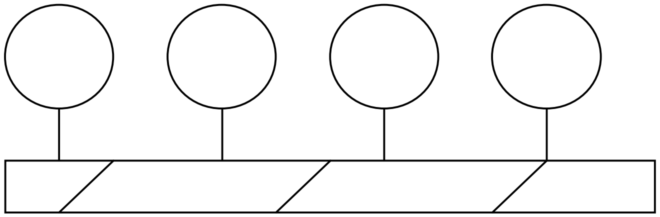
Stacks of Nanometer Thick Discs



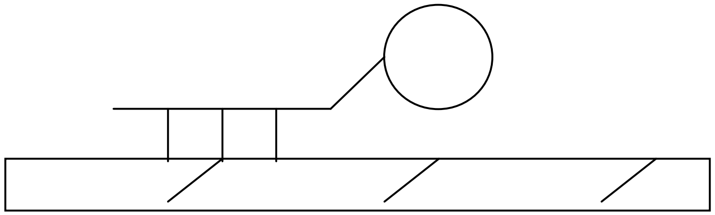
Enzymes are bound to solids by



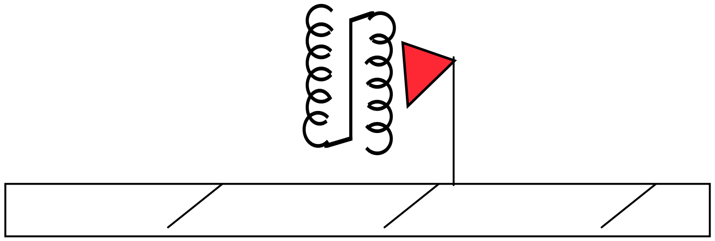
Adsorption/ionic binding



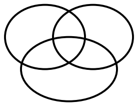
Covalent attachment



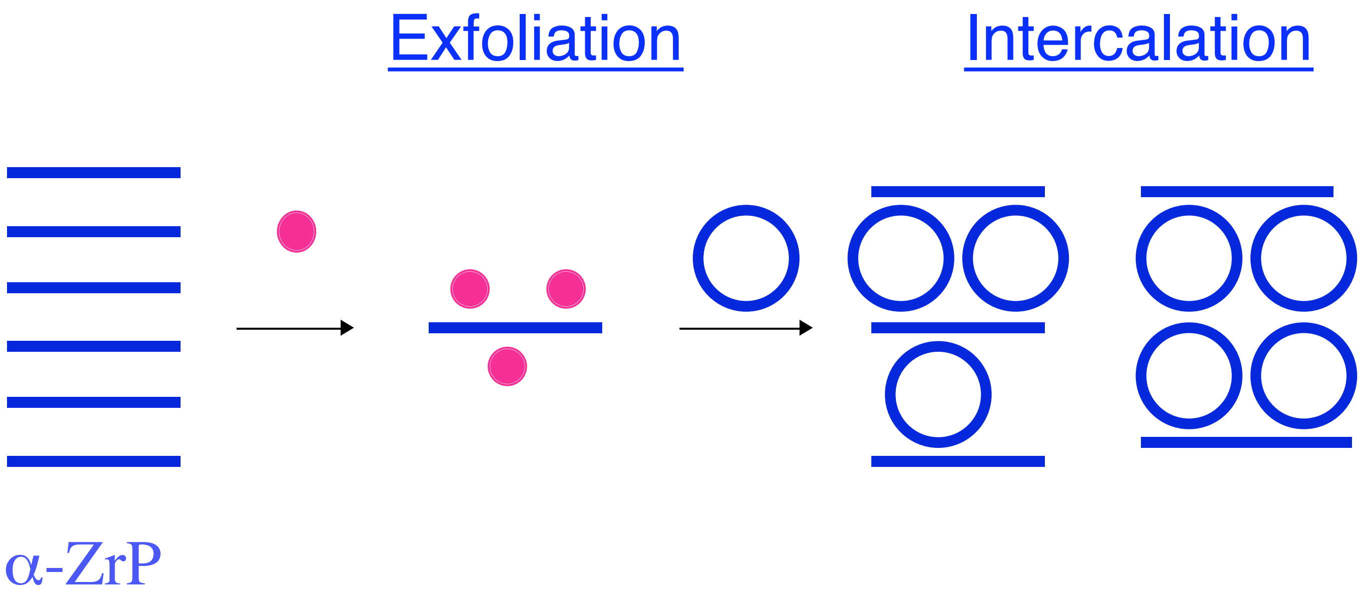
His-Tag binding



Bioaffinity binding



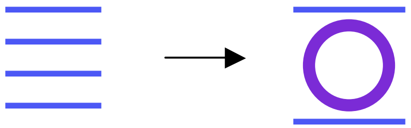
Spontaneous Protein Binding & Assembly



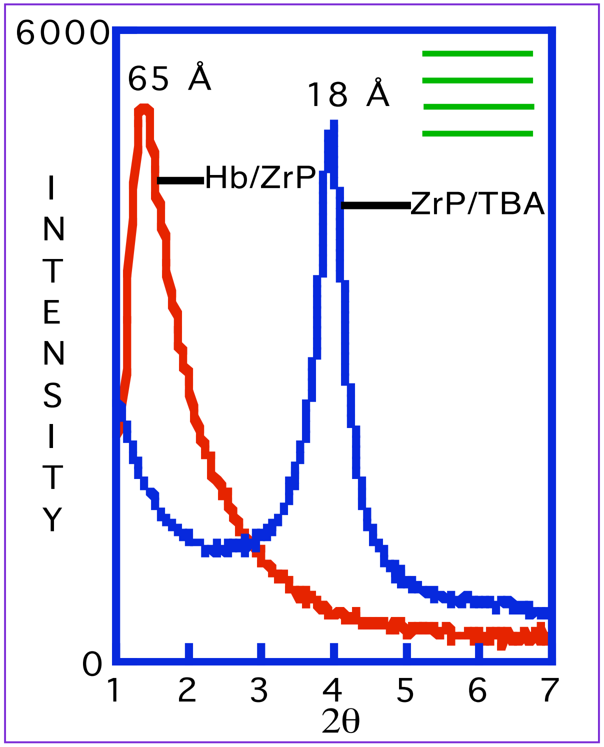
● = Tetrabutylammonium ion

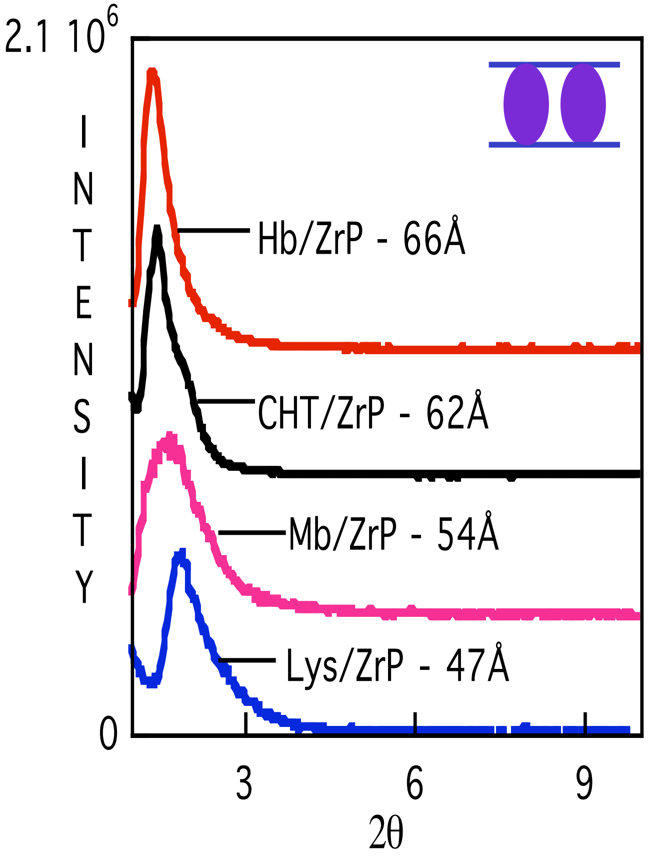
○ = Protein

Interlayer Spacings Increase on Intercalation



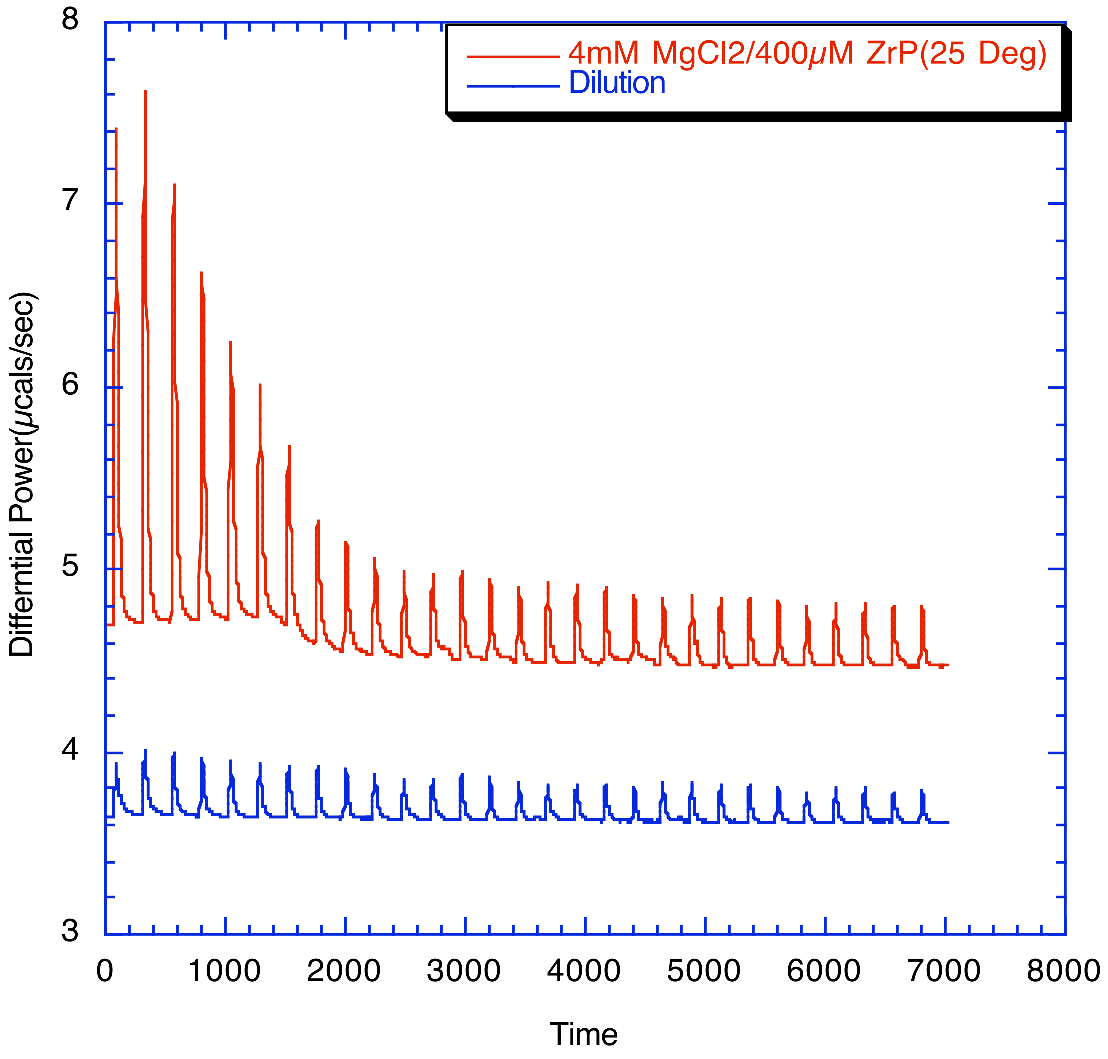
$n \lambda = 2 d \sin \theta$

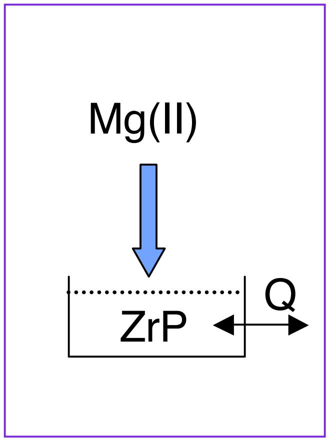




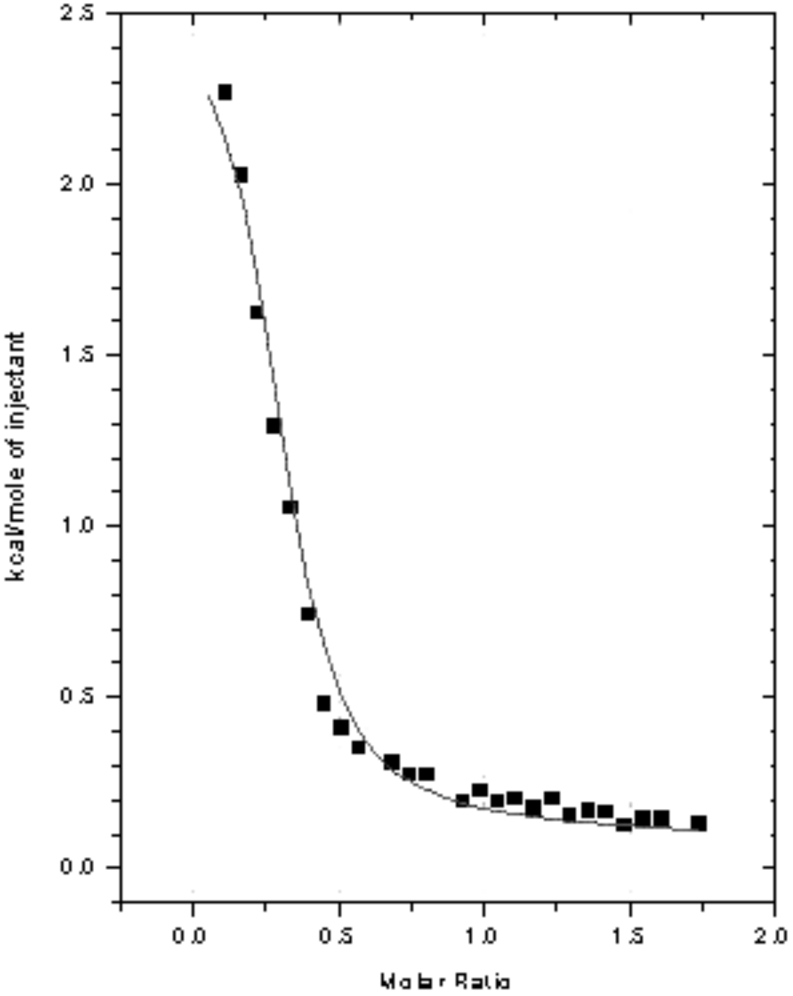
J. Am. Chem. Soc., 1999, 122, 830-837

Titration Calorimetry of Mg^{+2} Binding to ZrP





Thermodynamics of Mg^{+2} Binding to ZrP

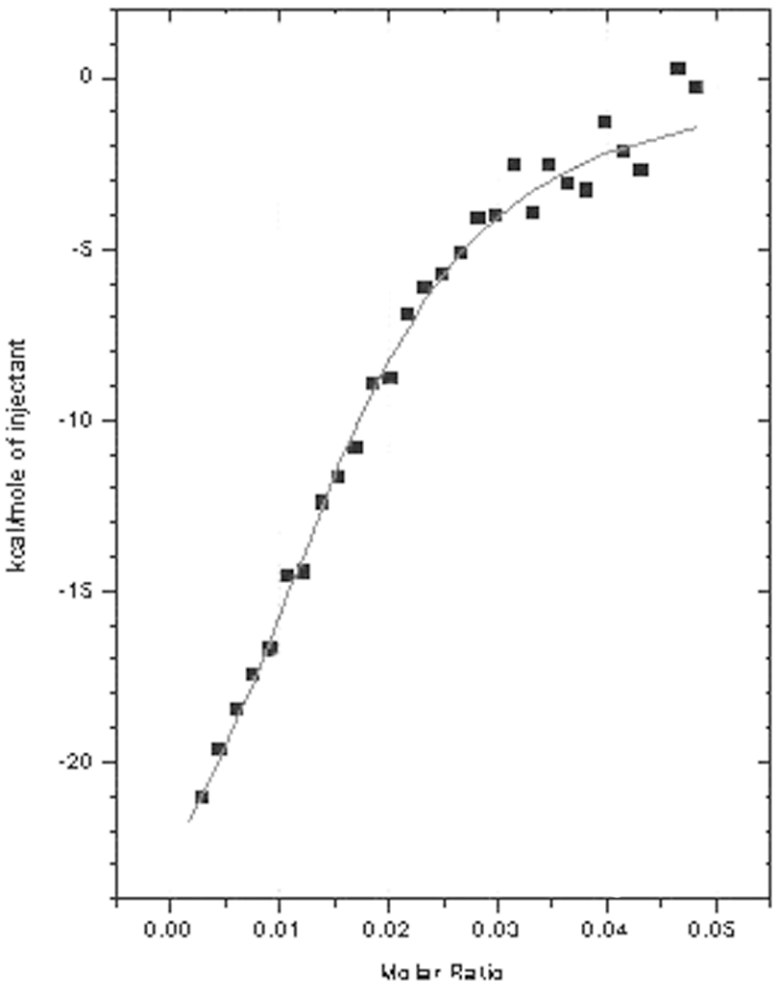


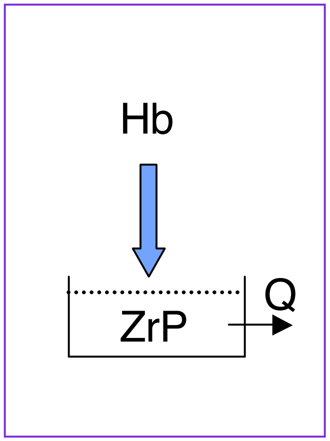
Two-site Binding Model

n_1= 0.3
K_1 = 6 E 4 M^{-1}
ΔH_1 = 2617+/- 47.6 J/mol
ΔS_1 = 31 e.u.

n_2 = 0.2
K_2 = 100 M^{-1}
ΔH_2 = 1.8 E4 +/- 2504 J/mol
ΔS_2 = 70 e.u.

Thermodynamics of Hb Binding to α-ZrP





Single-site Binding Model

$n_1 = 0.016$

$K_1 = 8 * 10^5 \text{ M}^{-1}$

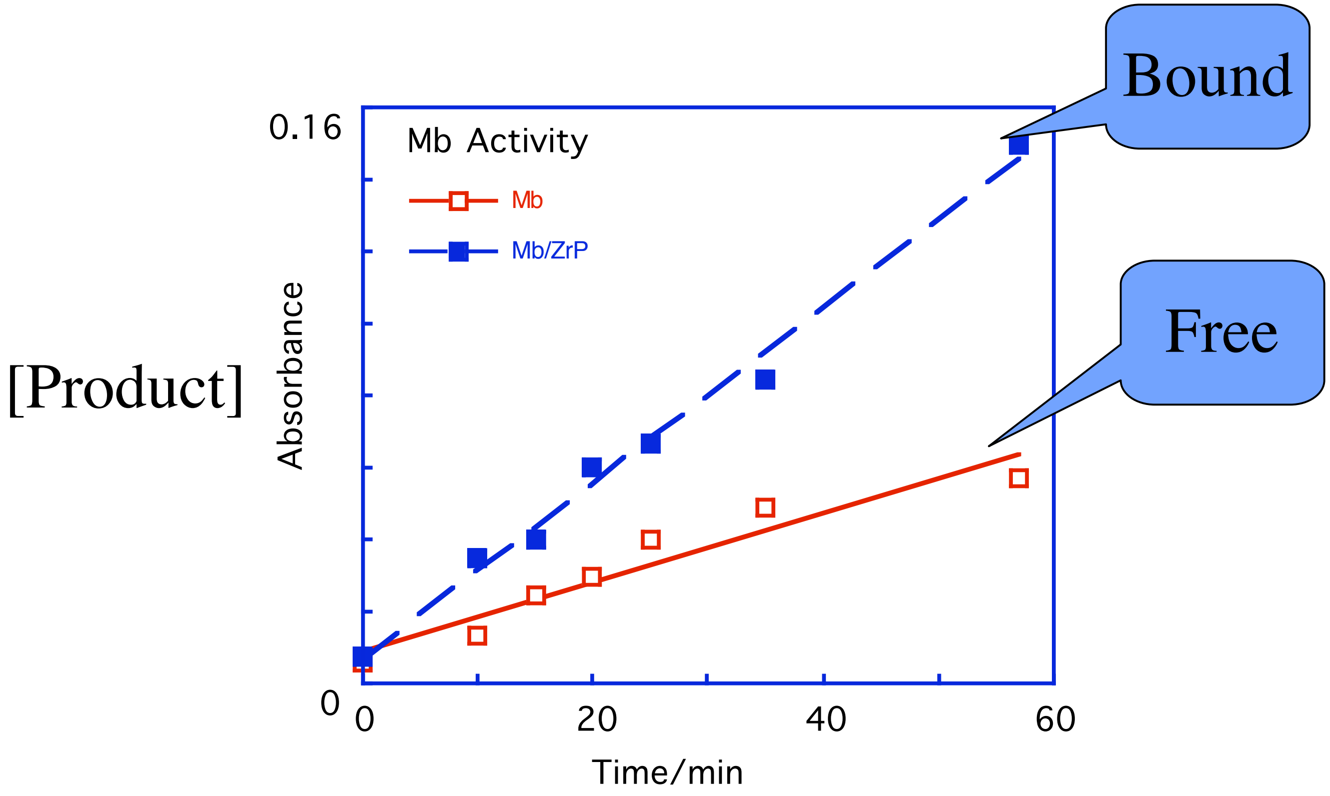
$\Delta H = - 2.9 * 10^4$ J/mol

$\Delta S = -70.43$ e.u.

Micropor. Mesopor. Mater., in Press

Enhanced Peroxidase-like Activity of Mb/ZrP

p-Methoxyphenol + Mb + H_2O_2 ---> Mb + Oxidized Product



J. Am. Chem. Soc., 1999, 122, 830-837

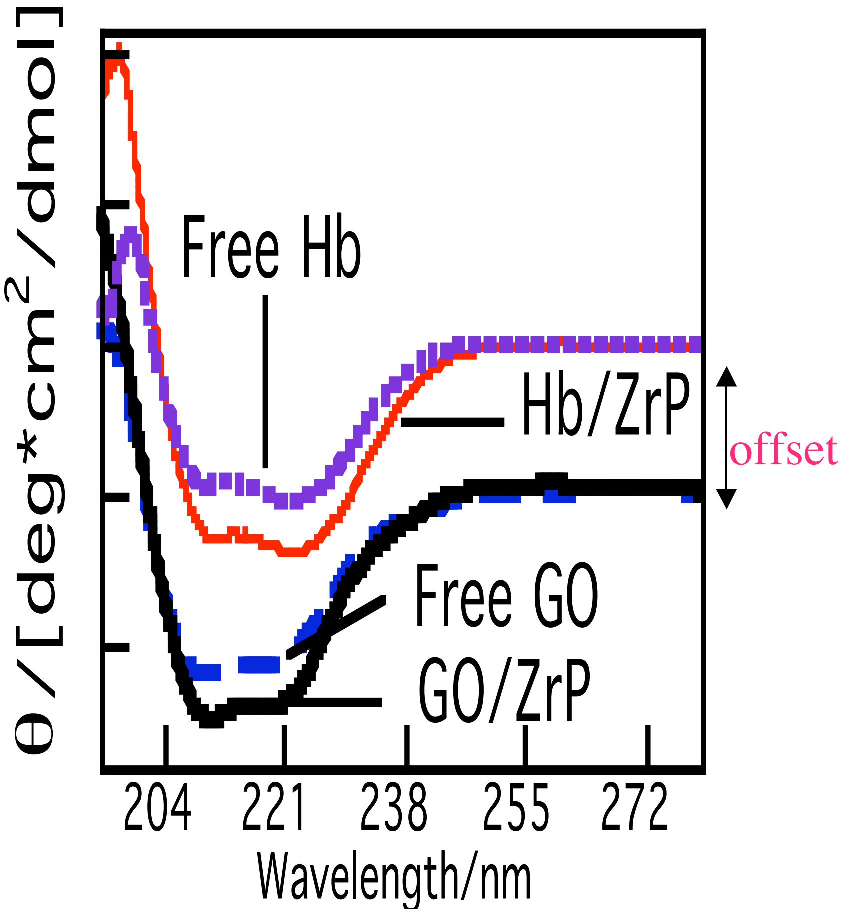
Retention of Activity in all Cases

Protein	Specific Activity	K_m	V_{max}
Mb	3.3 x 10^{-3}/s	1.4 mM	0.08 μM/s
Mb/α-ZrP	2.0 x 10^{-3}/s	1.6 mM	0.04 μM/s
Lys	11x10^{-3}/s	0.5 mM	0.63 μM/s
Lys/α-ZrP	9.4 x 10^{-3}/s	0.5 mM	0.63 μM/s
Hb	0.013/s	0.103 mM	34 nM/s
Hb/α-ZrP	0.038/s	0.112 mM	42 nM/s
CT	312/Ms		
CT/α-ZrP	400/Ms		
GO	2x10^{5}/Ms	0.8 mM	0.037mM/s
GO/α-ZrP	2.5 x 10^{5}/Ms	2.5 mM	0.042mM/s

$$\frac{1}{v_o} = \frac{1}{v_{max}} + \left(\frac{K_m}{v_{max}}\right)\frac{1}{[S]_o}$$

Secondary Structure is Retained

(Circular Dichroism)



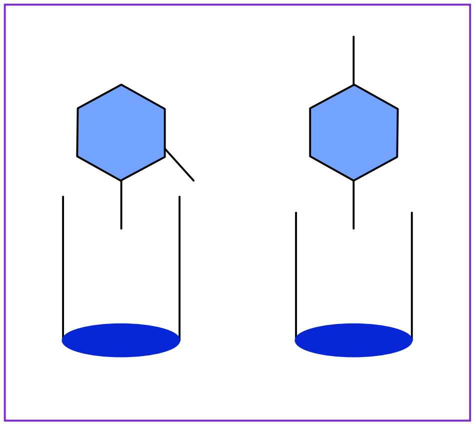
Protein	Amide I /Amide II band (cm^{-1})	CD peaks (nm)
Mb	1651/1530	210/222
Mb/α-ZrP	1651/1530	210/222
Lys	1650/1521	207 /223
Lys/α-ZrP	1650/1521	207 /223
Hb	1653/1521	210/222
Hb/α-ZrP	1653/1521	210/222
CT	1656/1523	202/231
CT/α-ZrP	1658/1523	202/231
GO	1645/1538	210/218
GO/α-ZrP	1645/1538	210/218

Improved Selectivity for Mb/α-ZrP

R-phenol + Mb + H_2O_2 ---> Mb + Oxidized Product

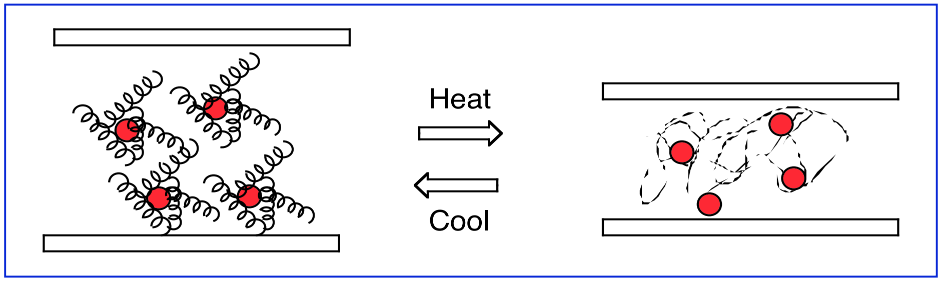
Substrate	Initial rate for Mb free/s^{-1}	Initial Rate for Mb/ α-ZrP/s^{-1}	Ratio of Rates bound/free	Oxidation Potential (V)
p-methoxyphenol	1.58 x 10^{-5}	4.00 x 10^{-5}	2.54	0.406
p-aminophenol	3.0 x 10^{-4}	6.0 x 10^{-4}	2.00	------
p-cresol	1.0 x 10^{-4}	----------	-----	------
o-methoxyphenol	1.83 x 10^{-3}	0.44 x 10^{-3}	0.24	0.456
phenol	1.33 x 10^{-4}	1.67 x 10^{-4}	1.25	1.04
o-cresol	2.92 x 10^{-3}	0.90 x 10^{-3}	0.31	0.556
aniline	1.70 x 10^{-4}	1.65 x 10^{-4}	0.97	0.70
m-aminophenol	0.9 x 10^{-4}	1.55 x 10^{-4}	1.72	------
o-aminophenol	3.16 x 10^{-3}	0.283 x 10^{-3}	0.09	0.124

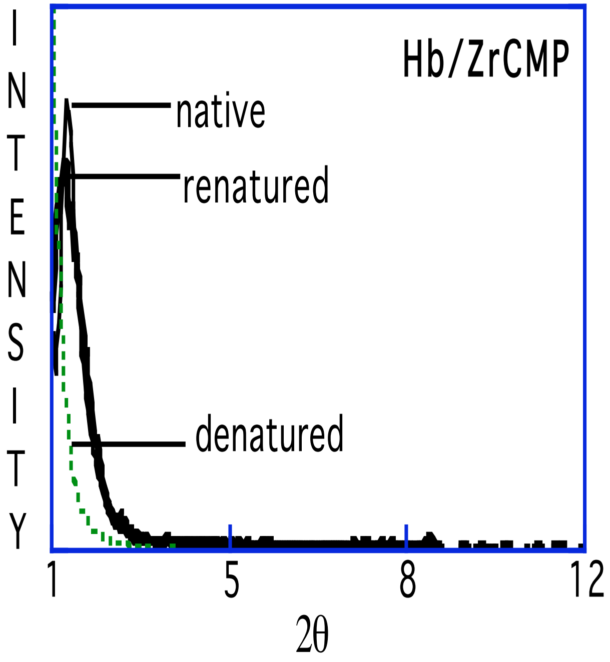
[Mb] = 5 μM, [substrate] = 6.6 mM, [H2O2] = 0.5 mM, buffer=10 mM K2HPO4 pH 7.2, errors <±10%

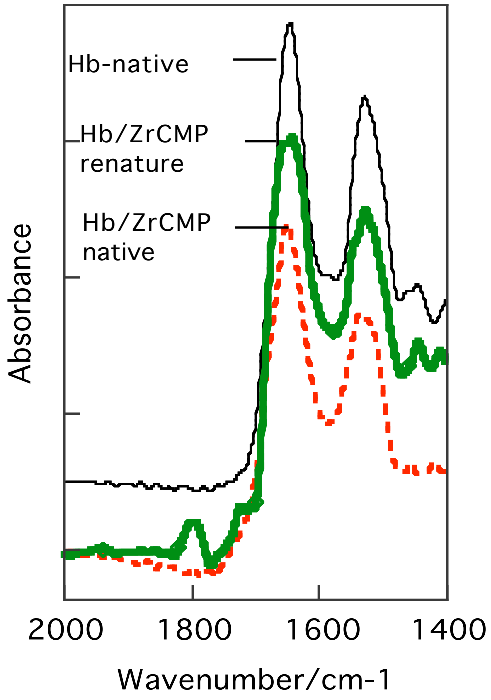


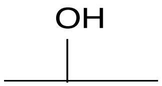
J. Am. Chem. Soc., 1999, *122*, 830-837

Reversible Thermal Denaturation of Hb on α-ZrCMP

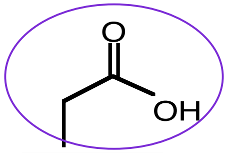




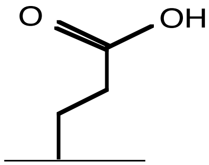




α-ZrP



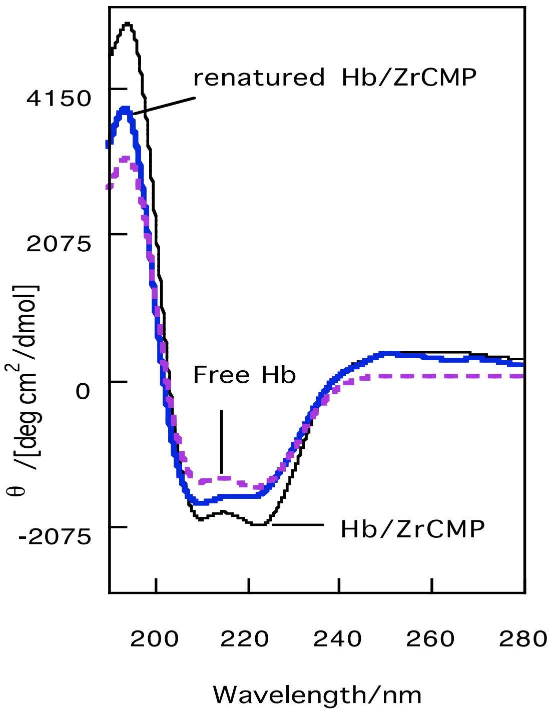
α-ZrCMP

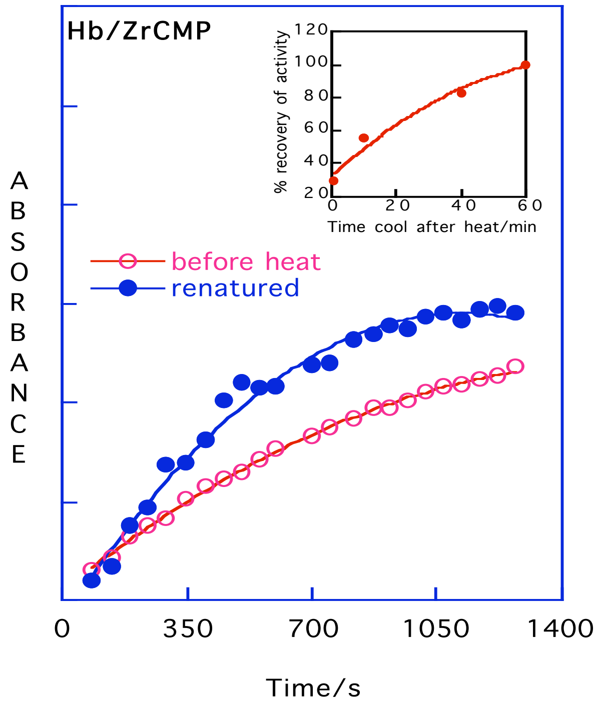


α-ZrCEP

Chem. Mater., 2001, *13*, 238-240

Reversible Denaturation of Hb on ZrCMP



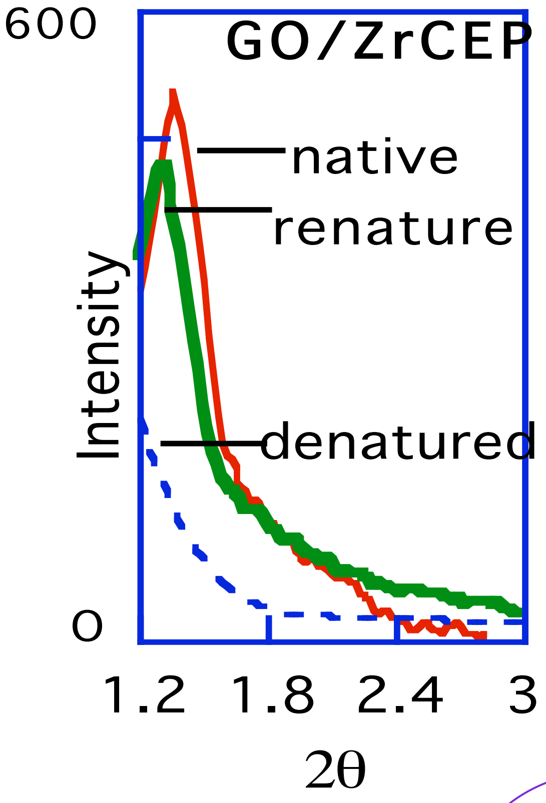


Sample	% recovery of α-helices	% recovery of activity
Hb	25	30
Hb/ZrP	0	50
Hb/ZrPAA	79	100
Hb/ZrCEP	NA	30

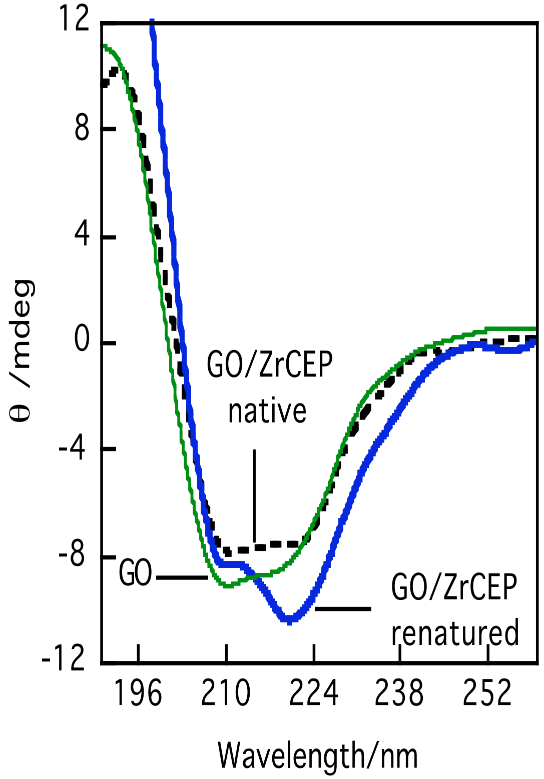
Submitted to MMM, 2004

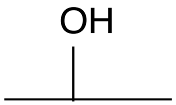
Resversible Denaturation of GO on α-ZrCEP

XRD

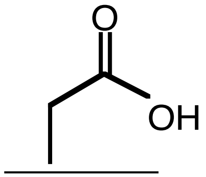


CD

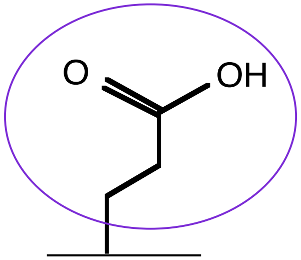




α-ZrP



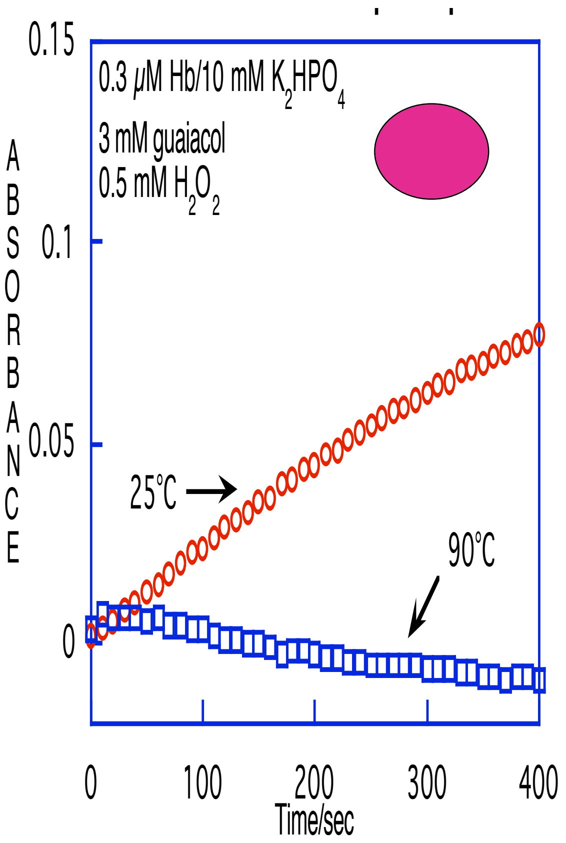
α-ZrCMP

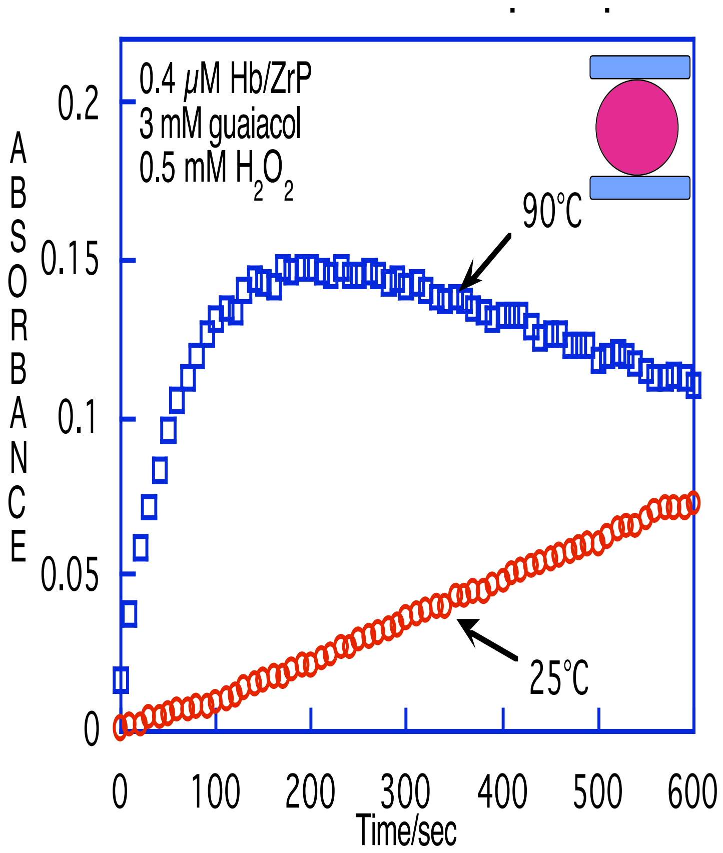


α-ZrCEP

Micropor.Mesopor. Mater. (2001) *47*, 407

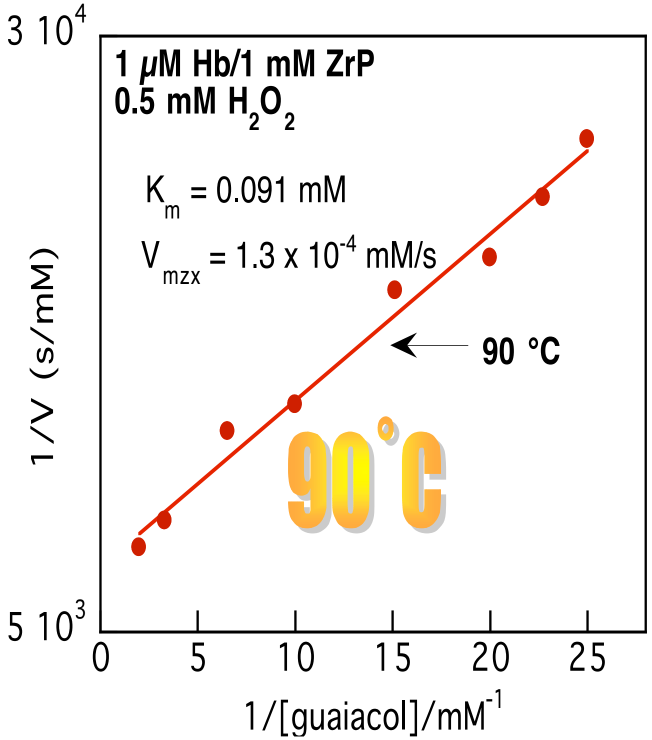
High Temperature Activities of HRP/Hb on ZrP

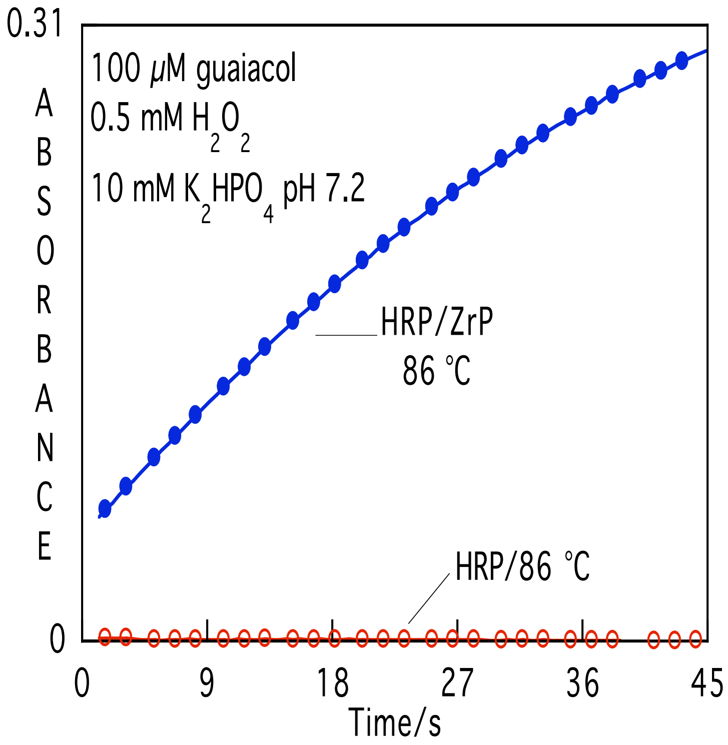




Chem. Commun., (2002) 2382

Vmax Increases ~4 fold at 90˚C

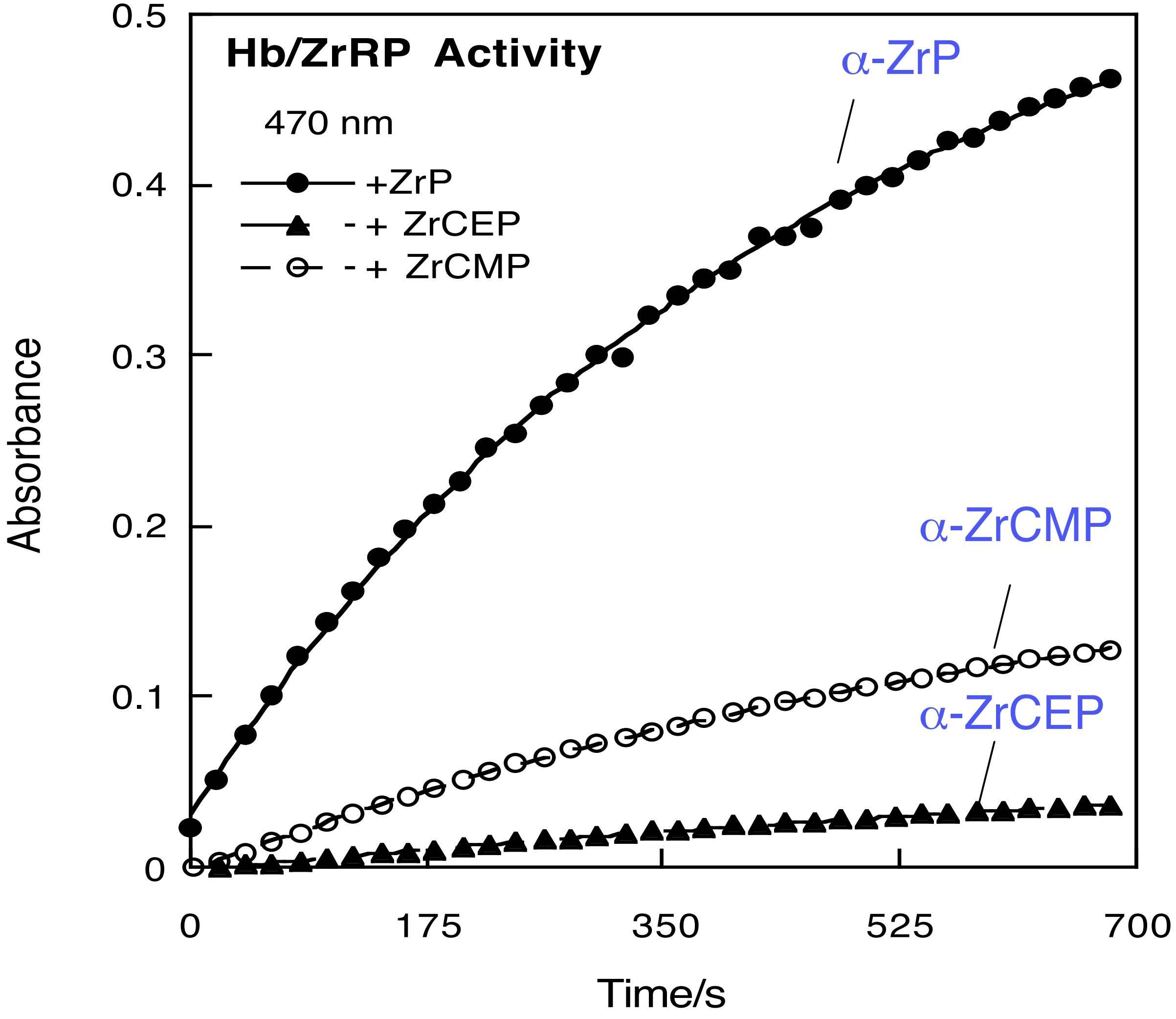




High T Activities of Hb and HRP

Sample/T	Hb	Hb/ZrP	HRP	HRP/ZrP	Mb/ZrP	HRP/ZrPMA
25 °C	0.35	0.45	3.6	1.8	0.14	10
90/86 °C	NA	1.6	NA	3.6	NA	0.9

Hb Activity is Modulated by Solid Surfaces

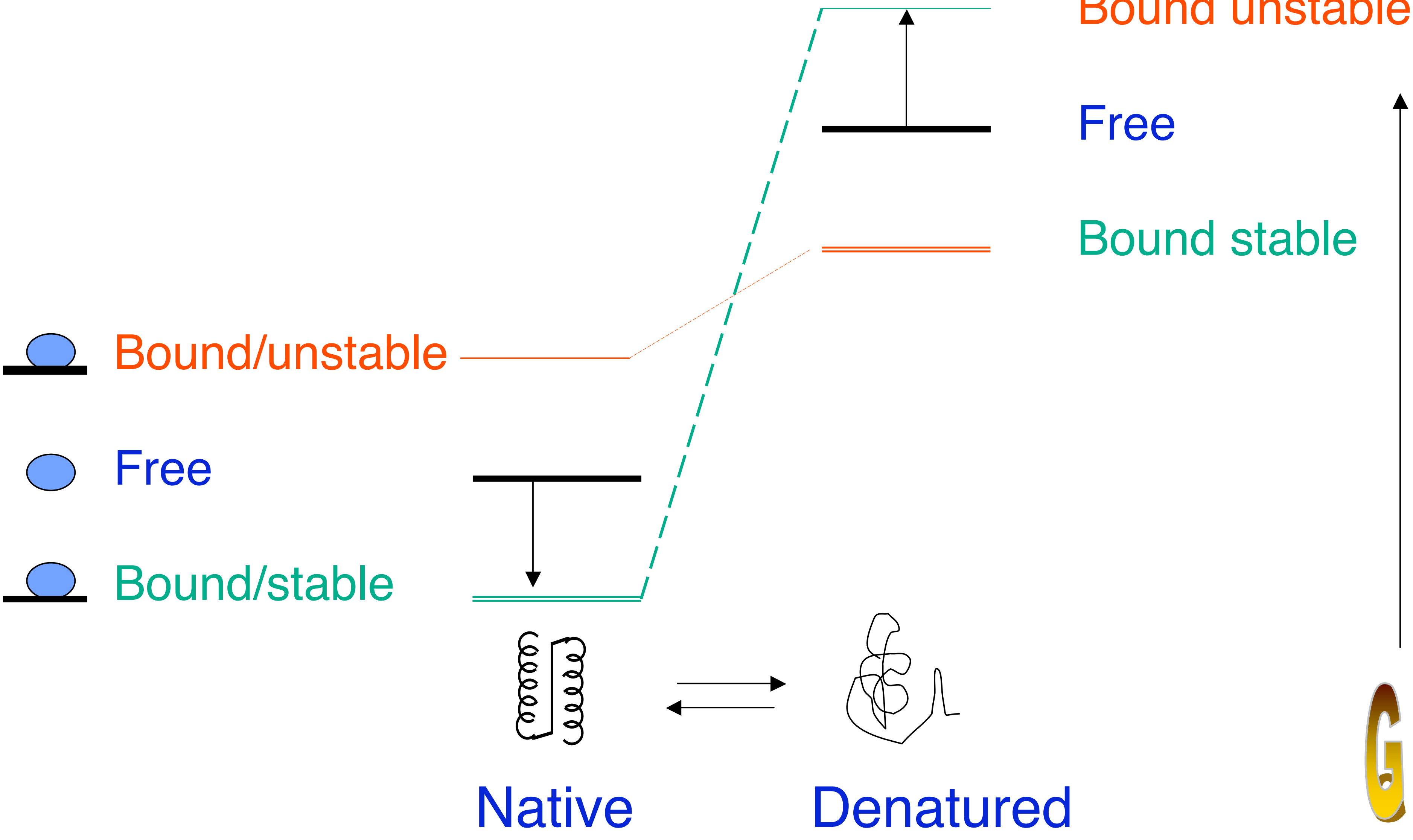


Conclusions

1. Proteins are intercalated and they retain structure.
2. Activity and selectivity (Mb/ZrP) are improved.
3. Hb/ZrCMP and GO/ZrCEP) show reversible thermal denaturation.

4. High temperature activities of HRP/ZrP, Hb/ZrP are demonstrated.
5. Protein folding/annealing (Mb/ZrCEP) is demonstrated.
6. Unique features of ZrP/phosphates are demonstrated.

How to Improve Enzyme Stability?



Bound unstable

Free

Bound stable

Bound/unstable

Free

Bound/stable

Native

Denatured

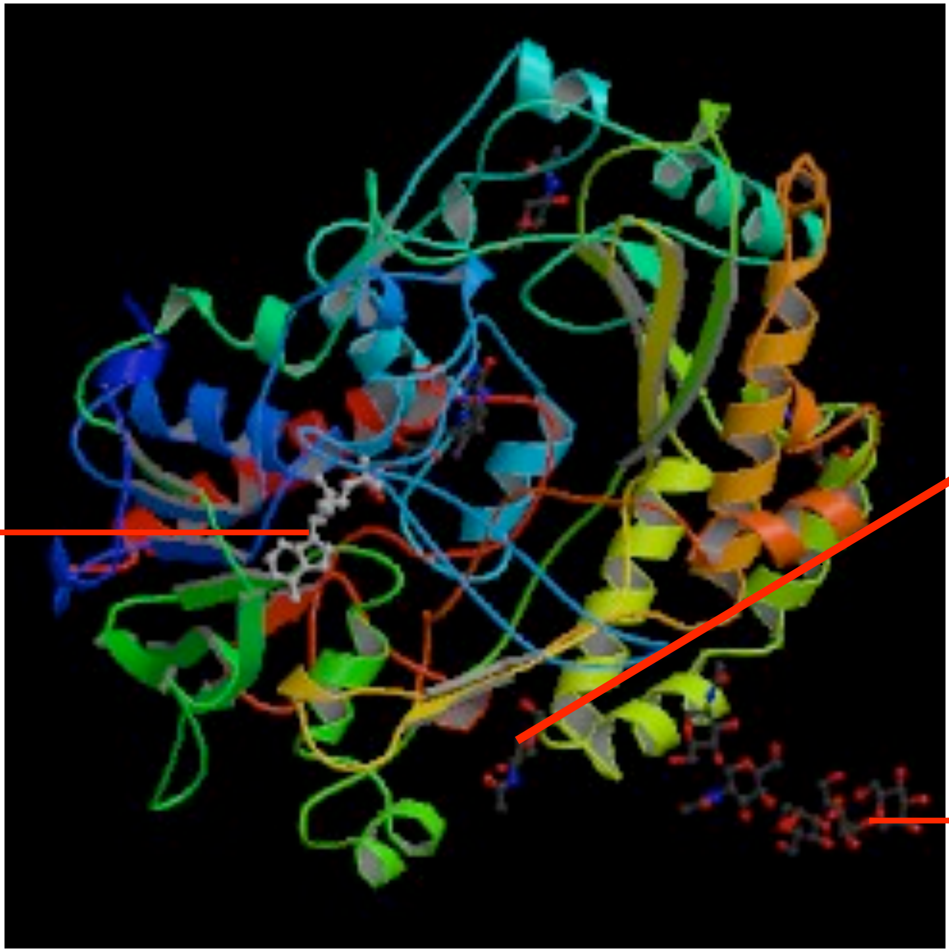
G

Advantages of Protein Encapsulation

1. High local concentrations of enzyme are achieved.
2. Ability to accept probes in either the polar or nonpolar phase.
3. Even enzyme properties are altered in desired ways.
4. To extend the useful lifetime of bound proteins by inhibiting their microbial degradation, hydrolysis and deamidation, while preserving their activity*(1).*

(1) Fang, J., Knobler, C. M., *Langmuir* **1996**, *12*, 1368, Mrksich, M., Sigal, G. B.,Whitesides, G. M., *Langmuir* **1995**, *11*, 4383. Kennedy, J. F., White, C. A., In *Handbook of Enzyme Biotechnology*, Wiseman, A. Ed., Ellis howood Lts., Chichseter, 1985, p 147.

Structure of Glucose Oxidase

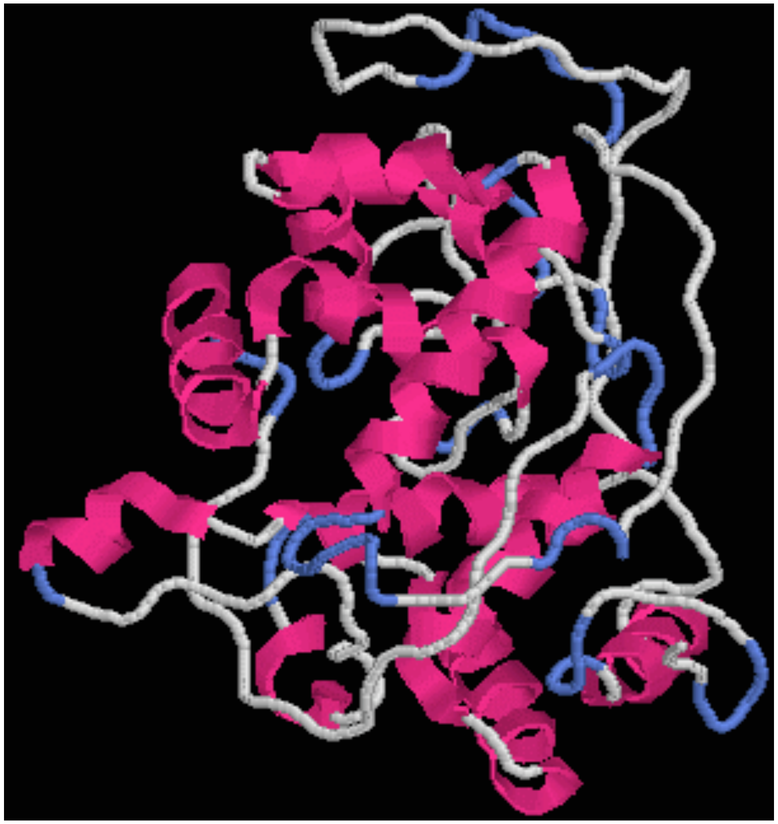


Flavin-Adenine
Dinucleotide

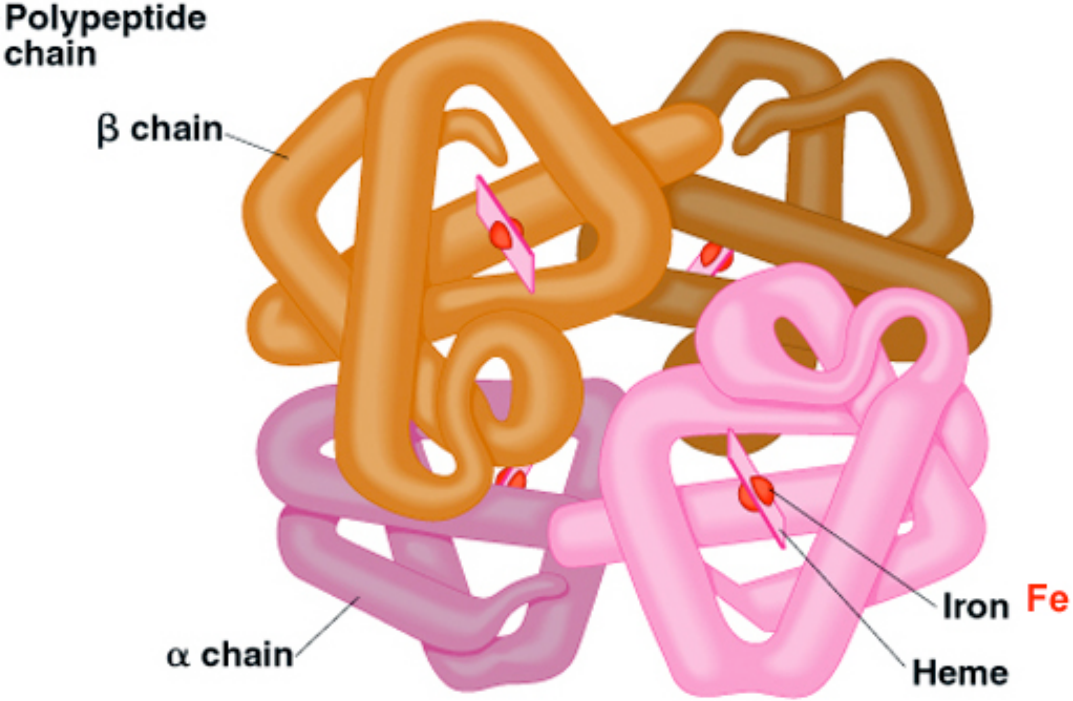
Alpha
D-Mannose

N-Acetyl
-D-Glucosamine

Structures of HRP & Hb



(a) Horseradish Peroxidase



(b) Hemoglobin

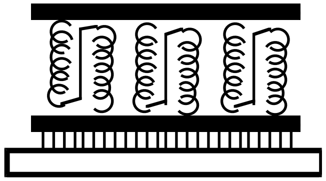
Goal

1. To study Protein-Surface interaction.

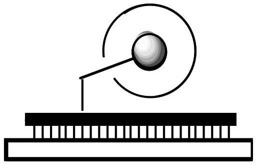
2. High temperature enzymology.

Specific Aims

1) Synthesis of protein-ZrP monolayer on gold.



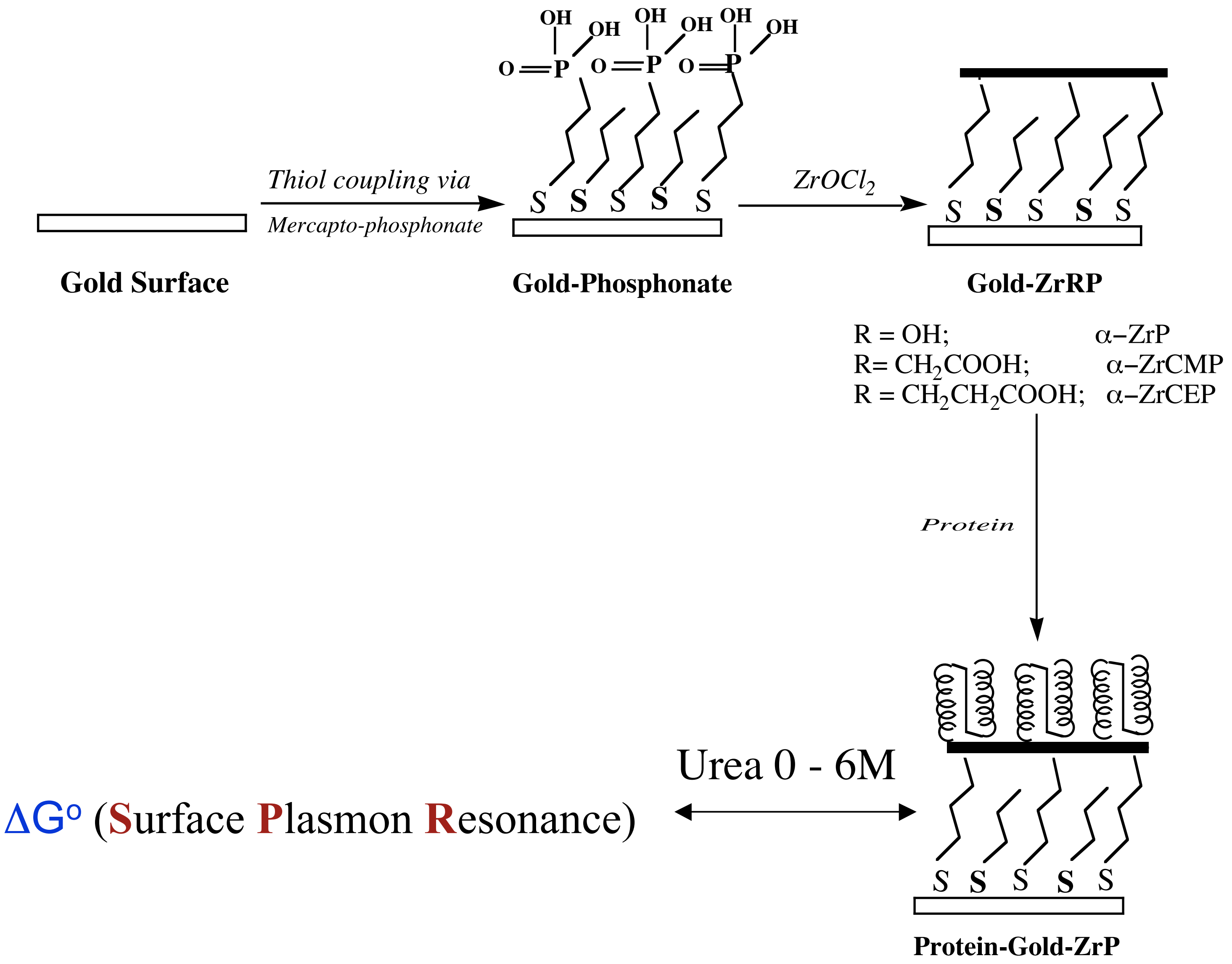
2) Attachment of Protein to the tethered heme.



3) Conformational, stability and activity studies on the two above mentioned surfaces.

4) Monitor cell binding to these solids and use it for pathogen detection.

Synthesis of protein-ZrP monolayer on gold

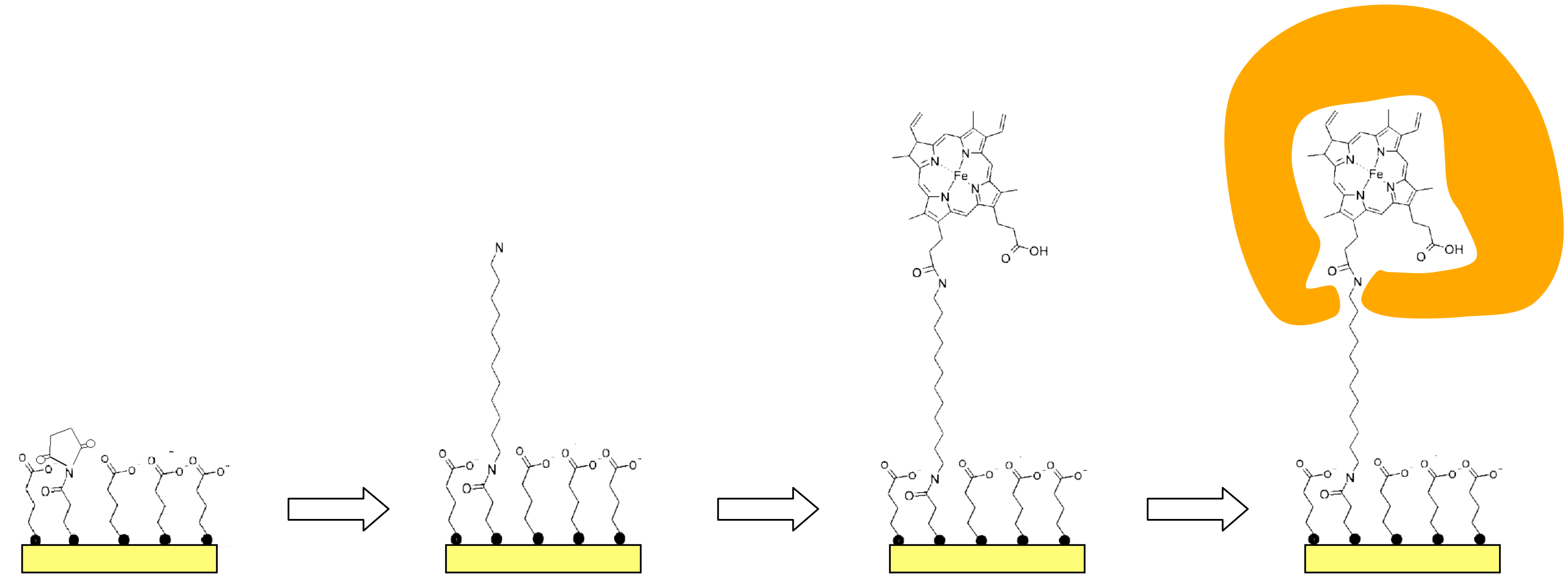


R = OH; α–ZrP
R= CH_2COOH; α–ZrCMP
R = CH_2CH_2COOH; α–ZrCEP

Urea 0 - 6M

ΔGº (**S**urface **P**lasmon **R**esonance)

Attachment of Protein to the tethered Heme



1:100 mixed SAMs

STEP 1

Linker attachment

STEP 2

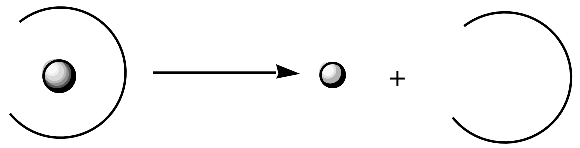
heme attachment

STEP 3

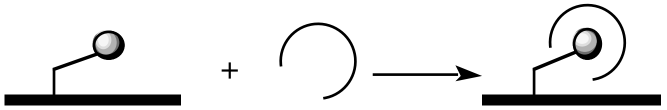
apo-protein binding

STEP 4

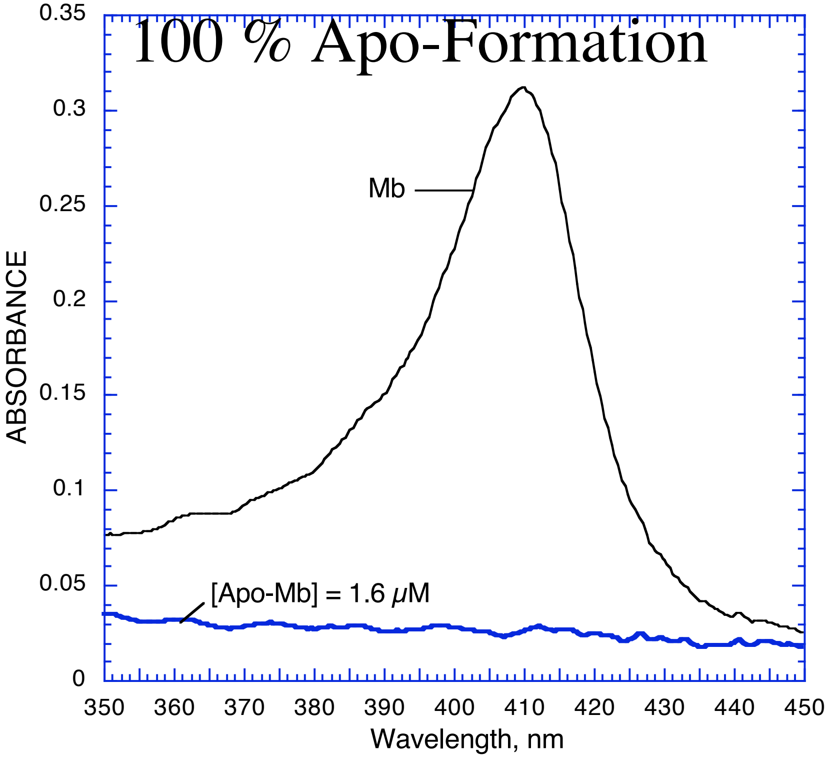
Reconstitution of Tethered Heme

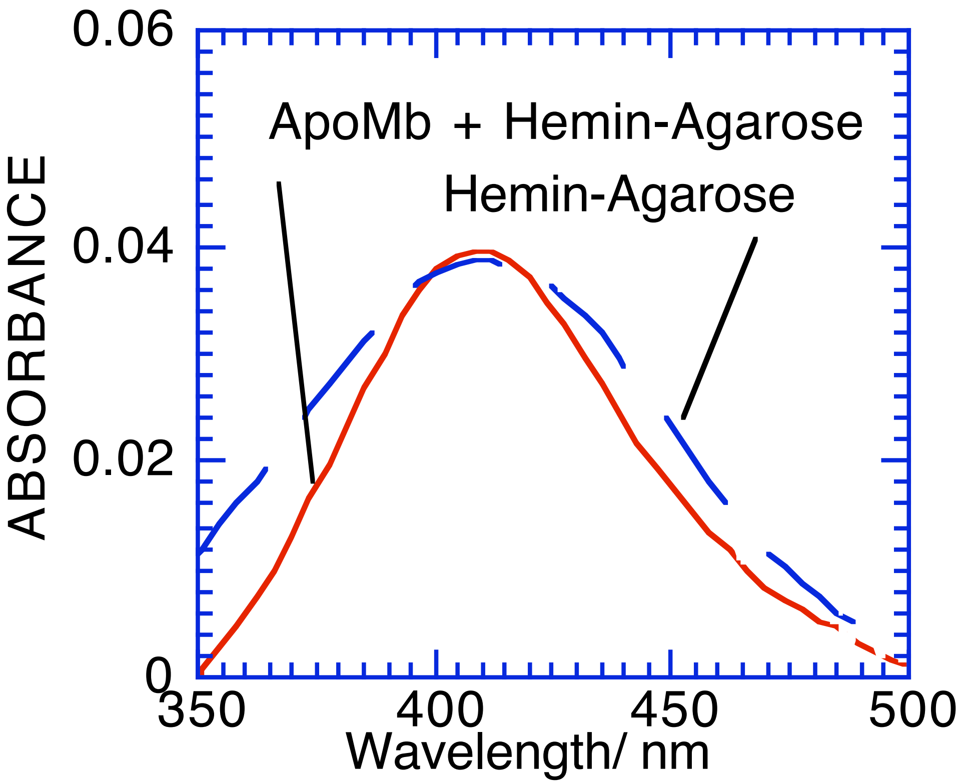


Heme-Protein **Heme** **Apo-Protein**

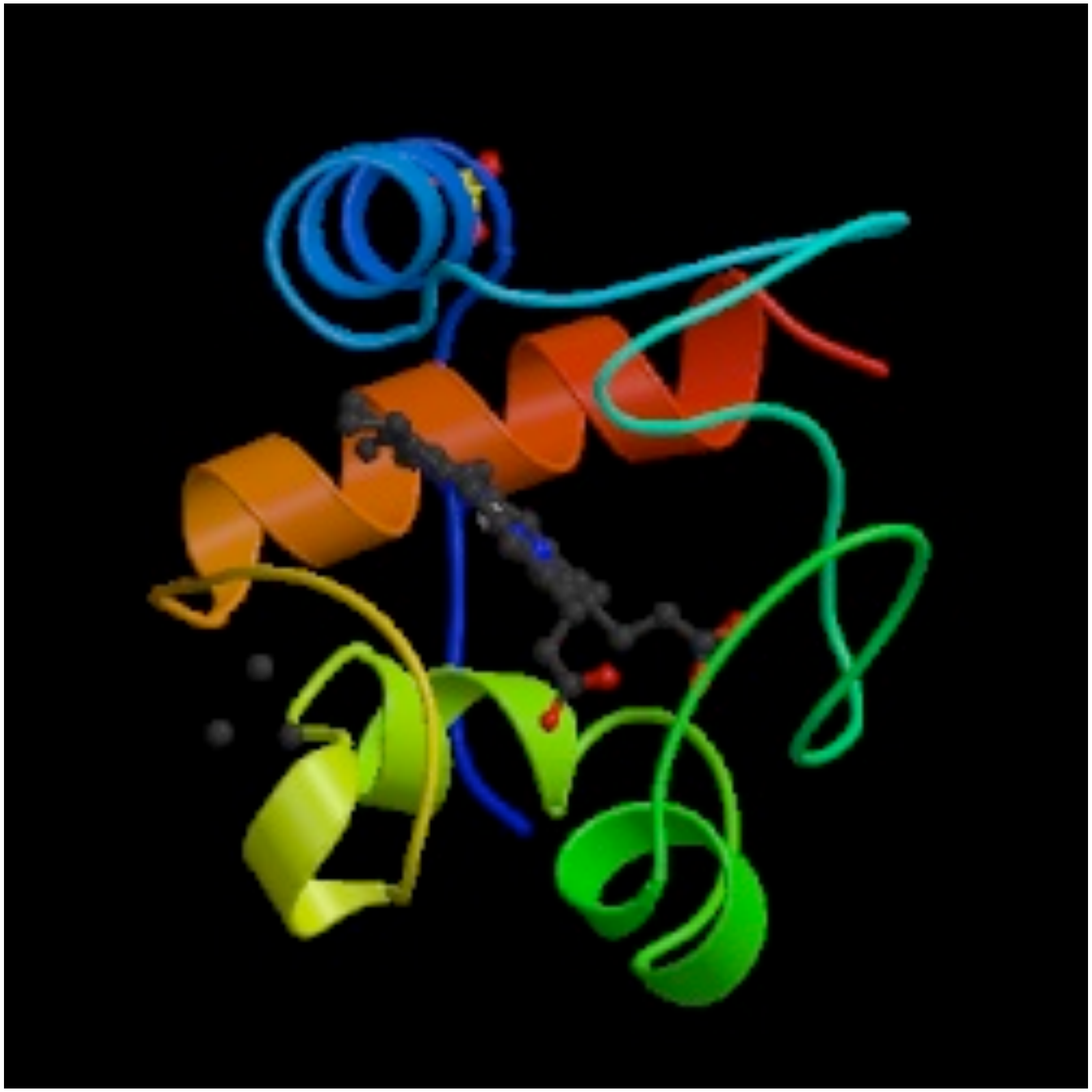


Hemin-agarose **Apo-Protein** **Reconstituted Protein**



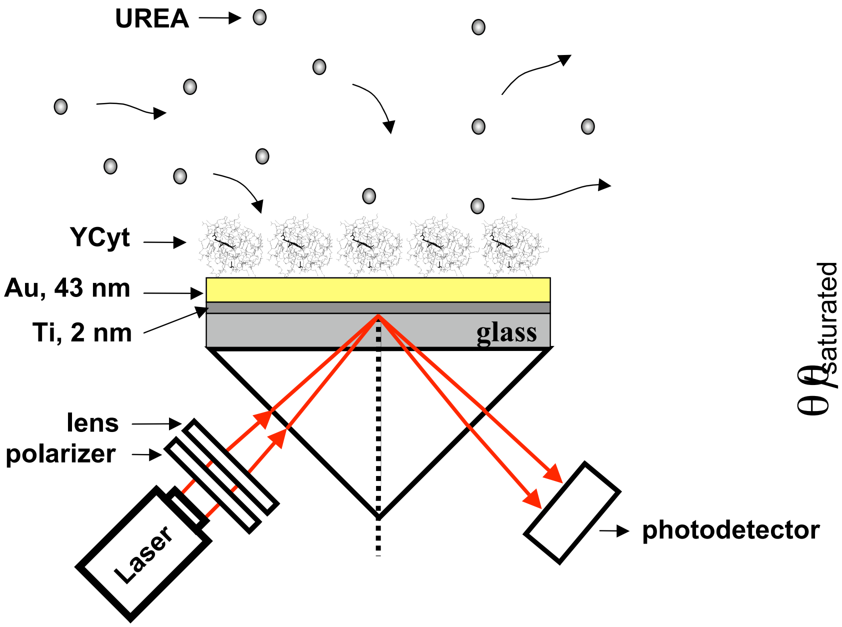


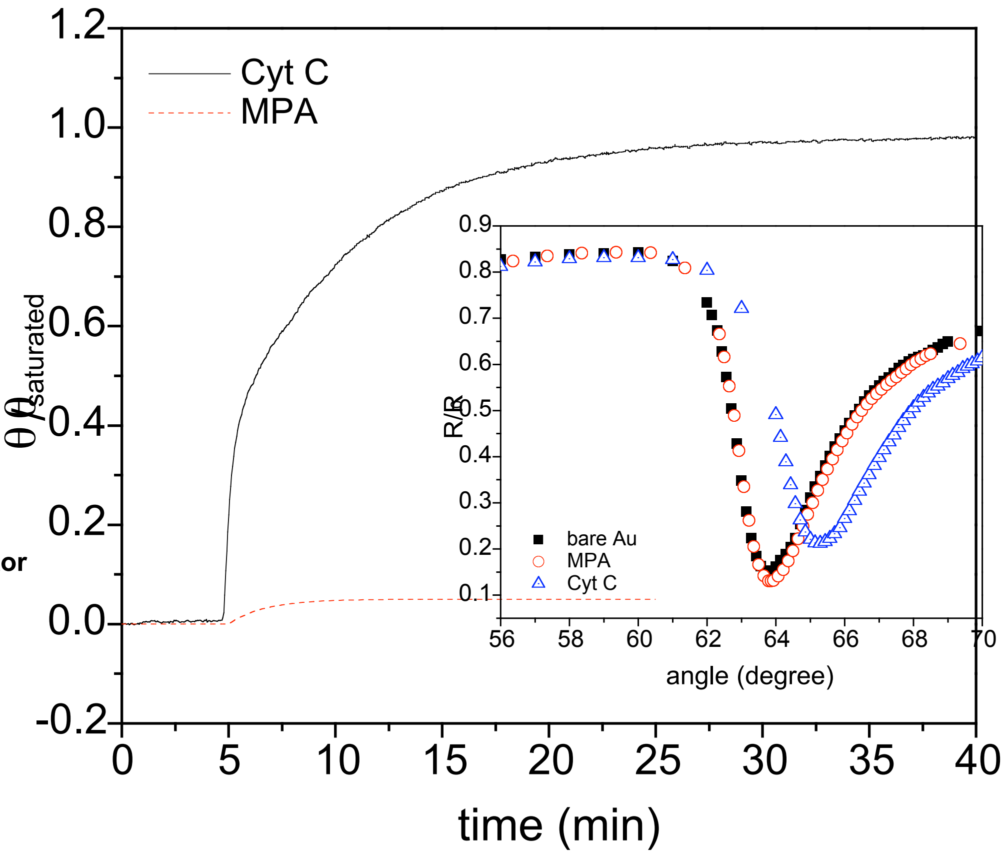
Yeast iso-1-cytochrome c (YCyt)



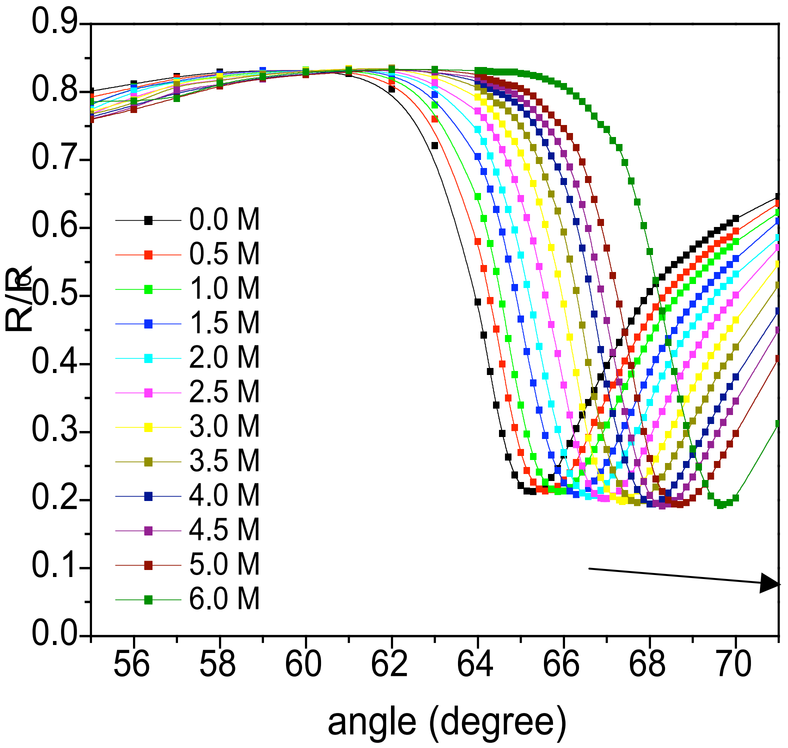
Surface Plasmon Resonance

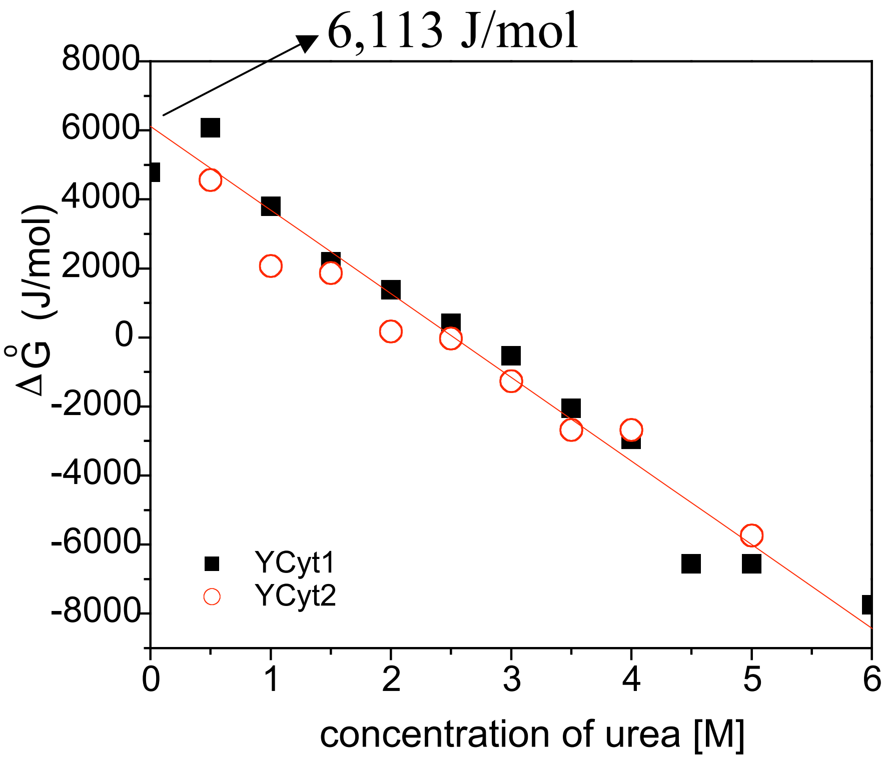
$$K_x = K_0 \eta_{inc} \sin(\Theta_{inc})$$





Denaturation of Cyt-C on Gold





ΔG^o of Cyt C in bulk solution = 27.2 kJ/mol

Ref. http://www.nature.com/nsb/journal/v6/n10/fig_ab/nsb1099_910_T1.html

Summary

1. Novel approach to study Protein-denaturation by SPR.

2. Reconstitution of tethered heme is demonstrated.

3. The denaturation of Cyt-C on gold is reversible.

4. ΔG^0 denaturation on Au << ΔG^0 in solution.

5. Protein-ZrP-Gold will provide further insight into protein surface interaction.

DST

$$\boxed{R_{cytC}/R_0 \approx 1 -(4\Gamma_{in}\Gamma_{rad}) / [(K_x - \Gamma^{r'})^2 +(\Gamma_{in} + \Gamma_{rad})^2]}$$

Γ_{in} =Imaginary$[K^0{}_x]$, Γ_{rad} = Imaginary$[\Delta K^{Au}{}_x]$, $\Gamma^{r'}$= Real $[K_x]$

$K_x = K_x{}^0 + \Delta K^{Au}{}_x + \Delta K_x{}^{cyt}$